# UNIVERSITEIT VAN PRETORIA

## FAKULTEIT VEEARTSENYKUNDE
## FACULTY OF VETERINARY SCIENCE

# 10th Faculty Day
# de Fakulteitsdag

October
Oktober

1, 1993

PROGRAM EN OPSOMMINGS
PROGRAMME AND SUMMARIES

SmithKline Beecham

**Fakulteit Veeartsenykunde, Universiteit van Pretoria**

**Faculty of Veterinary Science, University of Pretoria**

# TENTH FACULTY DAY

# TIENDE FAKULTEITSDAG

*1 October/Oktober 1993*

**Sponsored by/Geborg deur: SmithKline Beecham Animal Health Division, a Division of Smithkline Beecham Pharmaceuticals (Pty) Ltd**

*Reëlingskomitee/Organizing Committee*

Proff R.I. Coubrough, J.G. van der Walt, R.C. Krecek, B.L. Penzhorn, F.J.M. Verstraete, I.B.J. van Rensburg; Drr W.A. Schultheiss, E.A. Boomker; Mnrr F. Beukes, F.A. Nel

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# MESSAGE FROM THE DEAN

This year we celebrate our 10th Faculty Day. Certainly an achievement of which we can be justly proud! The central theme of our Faculty Days, to provide a forum for the presentation of research results, is firmly ensconced. Each year the nature of the material presented reflects an improvement on the previous year. An improvement brought about by a heightened commitment to the establishment of sound research bases in each academic department. The development of research themes, the wider use of research teams which involve workers from various disciplines within the Faculty as well as those from institutions elsewhere, have provided a greater depth in the research endeavour and is leading to the establishment of centres of expertise. This is essential for our Faculty to survive as an institution that can command international standing. The quality of the scientific presentations and posters presented this year augers well in this regard.

Faculty Day also provides a channel through which the diversity of the Faculty's talents can be expressed. This year several exciting demonstrations will be presented which focus on teaching aids and also show some of the highly sophisticated equipment which is available for our research programmes. Exhibits by each Department depict something of their activities, all of which contribute significantly to the stature of our Faculty.

The opening of our flagship, the Veterinary Academic Hospital (VAH), forms a focal point of our 10th Faculty Day programme. The VAH provides our Faculty with facilities for teaching, service and research which measure up to the best in the world. Used to the full they can only but give us a leading edge in our academic endeavours.

Prof Johnny van der Walt and his team have once again gone to great lengths to provide us with a really excellent scientific programme. This 10th Faculty Day has something for everyone and provides a shop window for the exposition of our research, service and teaching talents which must surely engender a spirit of pride in us all. To those who organise, to those who present research results or demonstrate departmental activities, to those who came to listen, learn, enjoy and support the day a sincere word of thanks. I have no doubt that what you experience cannot but fill you with a sense of fulfillment and satisfaction.

**PROF R I COUBROUGH**
**DEAN**

# PROGRAMME/PROGRAM

## FACULTY DAY 1 OCTOBER/OKTOBER 1993 FAKULTEITSDAG

07:45-08:15 **REGISTRATION AND COFFIE/REGISTRASIE EN KOFFIE**

08:15-17:15 **DEPARTEMENTELE UITSTALLINGS/DEPARTMENTAL EXHIBITIONS**

08:15-08:30 **WELCOME BY THE DEAN/VERWELKOMING DEUR DEKAAN**

08:30-09:45 **NAVORSINGSPROGRAM/RESEARCH PROGRAMME**

Sessie I/Session I Chairman: Prof B.L. Penzhorn

1. Development of A Soft-tissue Inflammation Model in Thoroughbred Horses. *C.R. Short, **A.J. Guthrie**, G.E. Swan, M.S.G. Mülders, V.M. Killeen & J.P. Nurton*

2. The rapid and specific detection of *Mycoplasma* spp in poultry by the polymerase chain reaction. ***R.R. Bragg***

3. Preliminary report on the parasites of the Laughing Dove (*Streptopelia senegalensis*). ***L.C. van Nieuwenhuizen**, A. Verster & R.A. Earle*

4. Vector-specific strains of *Babesia canis* in the ticks *Rhipicephalus sanguineus* and *Haemaphysalis leachi* in southern Africa. ***B.D. Lewis** & B.L. Penzhorn*

5. The immobilization of African lions using medetomidine and ketamine. ***D.G.A. Meltzer** & S.F. Quandt*

09:45-10:00 **"LECTURER OF THE YEAR" AWARD**
**TOEKENNING AAN "DOSENT VAN DIE JAAR"**

10:00-10:30 **PLAKKAATVOORSTELLING/POSTER PRESENTATION**

10:30-11:00 **TEA, POSTERS, DEMONSTRATIONS/TEE, PLAKKATE, DEMONSTRASIES**

**DEMONSTRATIONS**

1. Audiovisual Slide Bank - Veterinary Tropical Diseases
2. Computer-aided Case Studies - Department of Medicine
3. Equine Treadmill - Equine Research Centre

11:00-12:00 **INWYDING VAN VETERINêRE AKADEMIESE HOSPITAAL**
**INAUGURATION OF VETERINARY ACADEMIC HOSPITAL**

12:00-13:00 **HOSPITAL TOURS (for outside guests), DEMONSTRATIONS, POSTERS**
**HOSPITAALTOERE (vir buitegaste), DEMONSTRASIES, PLAKKATE**

13:00-14:00 **VINGERETE/FINGER LUNCH**

14:15-15:15 **NAVORSINGSPROGRAM/RESEARCH PROGRAMME**

Sessie II/Session II Chairman: Prof S.R. van Amstel

1. The usefulness of blood ammonia levels in the diagnosis of liver disease in dogs and cats. ***F. Reyers**, W.L. Berry, A.L. Leisewitz, H. Schroeder, L. Jacobson, R. Lobetti & R. Milner*

2. The effect of *Babesia canis* induced haemolysis on the canine haemoglobin oxygen dissociation curve. ***J.H. Taylor**, A.J. Guthrie, J.G. van der Walt, J. Janse van Rensburg & A.L. Leisewitz*

3. Water and electrolyte homeostasis in sheep without functional colons. ***R.A. Meintjes** & H. Engelbrecht*

4. Effect of water balance on nitrogen retention in sheep. ***J.G. van der Walt**, E.A. Boomker, R.A. Meintjes, W.A. Schultheiss, M. Grobler & H. Engelbrecht*

15:15-15:30 **VERVERSINGS EN BESIGTIGING VAN DEMONSTRASIES/PLAKKATE**
**REFRESHMENTS AND VIEWING OF DEMONSTRATIONS/POSTERS**

15:30-16:30 **NAVORSINGSPROGRAM/RESEARCH PROGRAMME**
Sessie III/Session III Chairman: Prof J.S.J. Odendaal

1. Survey of the designs of racehorse stables in the Pretoria, Witwatersrand and Vereeniging area of South Africa. ***R.J. Lund**, A.J. Guthrie & V.M. Killeen*

2. A prospective study comparing three methods of stabilising the stifle joint following cranial cruciate ligament rupture in dogs. ***G.L. Coetzee***

3. Pediculectomy for thoracolumbar spinal decompression in the Dachshund. ***A.M. Lubbe**, R.M. Kirberger & F.J.M. Verstraete*

4. The use of computer-aided image analysis for the objective evaluation of equine endometrial biopsies. ***C. Gerstenberg** & D.H. Volkmann*

16:30-16:45 **DEAN'S AWARD FOR BEST PAPER AND POSTER**
**DEKAANSTOEKENNING VIR BESTE REFERAAT EN PLAKKAAT**

16:45-16:55 **AFSLUITING/CONCLUSION**
Prof J.G. van der Walt

17:00-18:00 **SUNDOWNERS (for registered participants)**
**SKEMERDRANKIES (vir geregistreede deelnemers)**

# RESEARCH PROGRAMME/NAVORSINGSPROGRAM

## Short Communications/Kort Mededelings

### DEVELOPMENT OF A SOFT-TISSUE INFLAMMATION MODEL IN THOROUGHBRED HORSES†

*C.R. Short, A.J. Guthrie[1], G.E. Swan[2], M.S.G. Mülders[2], V.M. Killeen[1] & J.P. Nurton[1]*

Department of Pharmacology and Toxicology (Visiting Professor) and Department of Veterinary Physiology, Pharmacology and Toxicology, Louisiana State University, [1] Equine Research Centre, [2] Department of Pharmacology and Toxicology

The aim of this study was to develop and characterise a soft-tissue inflammation model in Thoroughbred horses.

Seven Thoroughbred mares had two subcutaneous tissue chambers surgically implanted on the lateral aspect of the neck. The body of the tissue chambers was constructed of a thermoplastic (Delrin®) and the open face covered with a silastic membrane which lay directly under the skin after insertion. This facilitated sampling of interstitial fluid via percutaneous needle puncture. The chambers were sterilized using ethylene oxide and were aseptically implanted while the horses were under general anaesthesia. Between 28 and 35 days, the stability and sterility of the chambers were evaluated. At least 42 days after insertion, inflammation was induced in one of the chambers by injection of 1 mℓ of a 1% carrageenan solution. Samples of the tissue fluid were collected from both chambers immediately prior to the injection of carrageenan, and at 4, 8, 12, 24, 36, 48 and 72 hours post injection. Skin temperatures over both chambers were measured just prior to the collection of each sample. The tissue fluid was collected for determination of cellularity; acid-base and gas status; and concentrations of total protein and albumin. The data were analyzed using a repeated measures analysis of variance.

When comparing the inflamed to the control chamber, a significant increase in cellularity and decrease in pH was observed from 4 through 48 hours. The albumin concentrations in the inflamed chamber were significantly increased from 8 through 72 hours. Carbon dioxide tension was significantly increased and bicarbonate concentration significantly decreased from 12 through 72 hours. Inflammation did not induce significant changes in oxygen tension, total protein concentration or skin temperature. The inflamed chambers also showed a mild swelling and increased sensitivity to tactile stimuli.

This study demonstrated that this model is reliable for producing a controlled mild local soft-tissue inflammatory reaction in horses. Our model may have extensive application for the *in vivo* evaluation of the effect of anti-inflammatory drugs.

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† Research project No. 36.5.83 approved by the Faculty Ethics and Research Committees.

# THE RAPID AND SPECIFIC DETECTION OF *MYCOPLASMA* SPP IN POULTRY BY THE POLYMERASE CHAIN REACTION

*R.R. Bragg*

Department of Poultry Diseases

*Mycoplasma gallisepticum* and *M. synoviae* cause very serious disease problems in the poultry industry. These organisms are often very difficult to detect as isolation and identification may take up to six weeks. The serological detection of these organisms, by rapid plate agglutination or enzyme linked immunosorbent assay (ELISA), is hampered by cross-reactions between *M. gallisepticum* and *M. synoviae*. Polymerase chain reaction (PCR) is a technique in which the DNA of the target organism is selectively replicated by the use of specific synthetically produced DNA primers. Once the target DNA of the organism has been replicated by PCR, the DNA can then be detected by either electrophoresis or enyme-labelled DNA probes. PCR kits for the detection of *M. gallisepticum* and *M. synoviae* are commercially available.

The primary aim of this study was to evaluate whether the South African isolates of *M. gallisepticum* and *M. synoviae* react with the DNA primers supplied with these kits. Furthermore, the extent to which the amplified DNA of South African isolates could be detected with the enzyme-labelled probes was investigated.

*M. gallisepticum* and *M. synoviae* isolates from seven and three different sites in South Africa, respectively, were assayed using the *M. gallisepticum* and *M. synoviae* kits. All of the South African isolates of *M. gallisepticum* and *M. synoviae* tested to date, have been detected by the commercially available PCR kits. These results indicate that no mutations in the DNA of South African isolates have occurred in the areas of genetic material which are recognised by the specific DNA primers used in the kits. The use of these PCR kits reduces the time required to confirm the presence of *M. gallisepticum* or *M. synoviae* in a flock from about 6 weeks to 36 hours.

# PRELIMINARY REPORT ON THE PARASITES OF THE LAUGHING DOVE (*STREPTOPELIA SENEGALENSIS*)†

*L.C. van Nieuwenhuizen, A. Verster & R.A. Earlé*

Department of Veterinary Tropical Diseases

The Laughing dove is the wild dove most closely associated with humans and human habitations in southern Africa. The seasonal prevalence of blood-, endo- and ecto-parasites of this bird are currently being studied. Laughing doves can also serve as reservoir hosts of various parasites which may infect cage birds. The survey started in July 1992 and will continue for two years.

By the end of July 1993, 290 birds had been examined for protozoa. The most common blood parasites were *Haemoproteus columbae*, which occurred in 36,6% of the birds, *Leucocytozoon marchouxi* in 17,6% and *Trypanosoma hannae* in 3,5%. This is the first record of *T. hannae* in Africa; it was last identified in India in 1935. Only 9% of the doves harboured more than one species of blood parasite. The prevalence of *H. columbae* was low during early summer, while the prevalence of *L. marchouxi* was high during late summer and that of *T. hannae* in July.

*Trichomonas gallinae* was present in 41,4% of the birds. Raptors are very susceptible to *Trichomonas* and may acquire infection when they prey on the doves.

Cestodes (mainly *Raillietina* spp.) were found at necropsy in all the doves. Four nematodes (*Dispharynx* sp.) were present in the proventriculus of one dove. Adult *Ascaridia columbae* was present in one at necropsy; eggs of this nematode were present in the faeces of another bird.

Low numbers of ectoparasites were present during the late winter months but infection rates increased as the summer progressed. *Pseudolynchia canariensis*, the vector of *H. columbae,* was found on 10,4% of the birds.

† Research project No. 36.5.80 approved by the Faculty Ethics and Research Committees.

# VECTOR-SPECIFIC STRAINS OF *BABESIA CANIS* IN THE TICKS *RHIPICEPHALUS SANGUINEUS* AND *HAEMAPHYSALIS LEACHI* IN SOUTHERN AFRICA[†]

*B.D. Lewis & B.L. Penzhorn*

Department of Veterinary Tropical Diseases

Experiments by Uilenberg and co-workers on cross-immunity studies and the results of Indirect Fluorescent Antibody tests indicated that strains of *Babesia canis*, the causative agent of canine babesiosis, from France, North Africa and South Africa were specific to three different tick vectors, *Dermacentor reticulatus*, *Rhipicephalus sanguineus* and *Heamaphysalis leachi* respectively. These experiments did not include transmission trials with the different vectors.

In southern Africa, two of these vector species occur, *Haemaphysalis leachi*, the yellow dog tick and *Rhipicephalus sanguineus*, the kennel tick. Strains of southern African *B. canis* are being investigated for vector specificity to these tick species. A strain of *B. canis* transmissible by all three instars of *Haemaphysalis leachi* has been isolated and tested for vector specificity.

Repeated attempts to transmit the strain with different instars of *Rhipicephalus sanguineus* have shown that although *R. sanguineus* is a known vector of *B. canis* in North Africa and Europe, it is apparently incapable of transmitting this southern African *H. leachi* derived strain. Attempts to find a southern African strain of *B. canis* that is transmissible by *R. sanguineus* have so far been unsuccessful, suggesting that this tick species may only be a minor vector of canine babesiosis in the region.

Further trials are continuing to confirm the presence of vector specific strains of *B. canis* in southern Africa.

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[†] Research project No. 36.5.31 approved by the Faculty Ethics and Research Committees.

# THE USE OF KETAMINE AND MEDETOMIDINE HYDROCHLORIDE FOR THE IMMOBILIZATION OF AFRICAN LIONS†

*D.G.A. Meltzer & S.F. Quandt*

Department of Veterinary Tropical Diseases (Price Forbes Chair in Wildlife Diseases)

Medetomidine is a novel α2-agonist which is more potent than xylazine. The effects of the drug can be reversed by the administration of atepamezole or yohimbine. This reversal suggested that this drug may be used in combination with ketamine hydrochloride for the immobilization of lions. The most commonly used drug combination for this purpose is a combination of tiletamine and zolazepam (Zoletil, Palmvet, JHB). Though very effective, the effect of zoletil cannot be reversed, leading to long recovery times, during which the animals have to be protected until they are able to fend for themselves.

Free-ranging lions were lured to a carcass by broadcasting sounds of lions and hyaenas at a kill. Lions were assigned at random to one of the three treatment groups and darted with either medetomidine and ketamine, zoletil or phencyclidine. The latter group of lions were captured with phencyclidine, which has a prolonged effect, so that pharmokinetic studies could be conducted. Immobilized lions were examined clinically. Heart and respiration rates and rectal temperatures were measured at 15 min intervals. Arterial blood samples were collected by needle puncture from the femoral artery as soon a possible after immobilization as well as from the femoral or a branch of the median artery, after insertion of a suitable catheter. Blood pressure changes after the administration of medetomidine were measured in the lions immobilized with phencyclidine.

The time intervals of ketamine/medetomidine and zoletil immobilization were similar. However, animals from the ketamine/medetomidine group appeared to be more relaxed and the drug effects could be reversed very effectively. Blood gas data did not differ between groups. The administration of medetomidine to animals immobilized with phencyclidine was followed by a rapid, marked increase in blood pressure with accompanying bradycardia.

The combination of ketamine/medetomidine is an effective drug combination for the immobilization of lions. The animals in this group were more relaxed than those immobilized with zoletil or phencyclidine. Both atepamezole and yohimbine were effective in reversing the effects of medetomidine and lions were able to walk away approximately 6 min after administration of either of these two antidotes.

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† Research project No. 36.5.16 approved by the Faculty Ethics and Research Committees.

# THE USEFULNESS OF BLOOD AMMONIA LEVELS IN THE DIAGNOSIS OF LIVER DISEASE IN DOGS AND CATS

*F. Reyers, W.L. Berry, A.L. Leisewitz, H. Schroeder, L. Jacobson, R. Lobetti & R. Milner*

Department of Medicine

Blood ammonia in fasting patients and in patients given an oral or rectal dose of ammonium salts (tolerance test) has been advocated as a sensitive and specific test for the diagnosis of porto-systemic vascular shunting (PSS) in humans, dogs and cats. Most of the published work confirms the sensitivity of the test but very little has been published in support of the specificity in the presence of other types of liver disease.

In this retrospective study of 153 dogs and 11 cats, the fasting ammonia concentration (all cases) and tolerance test (57 cases) were compared with serum enzymes, albumin, urea and bile acid levels in terms of a classification based on histopathology, ultrasonography, contrast portograms and clinical appraisal. The classes were acute hepatopathy (n=32), chronic hepatopathy/fibrosis (without evidence of PSS) (n=23), PSS (n=21), secondary liver disease (n=31), non-liver disease (n=52) and uncertain liver status (n=5). Multi-range comparisons (Scheffe) and Bayesian analyses (Galen and Gambino) were conducted.

It was found that the fasting ammonia and ammonium tolerance test were in fact sensitive for PSS but could not reliably distinguish PSS from other classes of liver disease (in particular chronic hepatopathy/fibrosis). There were no combinations of tests which could improve significantly on this specificity.

It may be concluded that the fasting ammonia and ammonium tolerance is not as specific for PSS as the literature would suggest. As other laboratory tests, alone or in combination with ammonia, could not improve on the diagnostic specificity, other diagnostic procedures such as histopathology or imaging techniques remain essential in the confirmation of PSS.

# THE EFFECT OF *BABESIA CANIS* INDUCED HAEMOLYSIS ON THE CANINE HAEMOGLOBIN OXYGEN DISSOCIATION CURVE†

*J.H. Taylor, A.J. Guthrie[1], J.G. van der Walt, J. Janse van Rensburg & A.L. Leisewitz[2]*

Department of Physiology, [1] Equine Research Centre, [2] Department of Medicine

Canine babesiosis is extremely common during the summer months in South Africa. Little research has been done to ascertain the effect of the disease process on the function of the remaining haemoglobin. This was the objective of this investigation.

Samples of blood were collected from normal healthy (n=7) and *Babesia canis* infected dogs (n=7). The blood was exposed to varying gas mixtures in a tonometer, after which the samples were subjected to blood gas analysis and haemoximetry. These data were used to plot haemoglobin oxygen dissociation curves (ODC) for both groups of dogs. The effects of various ligands of haemoglobin, including the effects of pH and $pCO_2$, were investigated. A state of acidosis and hypercapnia was simulated, and the response of the ODC to acidosis and hypercapnia compared. Values derived when simulating an acidosis and hypercapnia differed significantly from each other, falling outside a 95 % confidence interval. The data were used to plot ODC's for normal and *B. canis* dogs. The ODC of the *B. canis* infected group showed a decreased ability to shift to the right.

In a second experiment, blood was drawn from 7 healthy dogs, and split into two aliquots. Bilirubin, at 10 mg/d$\ell$, was added to one aliquot. This aliquot was compared, in a crossover trial, using the same tonometry/blood gas model and data analysis described above, to the other aliquot. The addition of bilirubin caused a significant ($p < 0,05$) shift to the right under acidotic conditions.

The results suggest that the haemoglobin from healthy dogs appears to react differently as an oxygen carrier in the presence of $H^+$ and $CO_2$, to haemoglobin taken from dogs suffering from babesiosis. This decreased Bohr effect probably exacerbates the severe tissue hypoxia associated with this disease by impairing the ability of haemoglobin to off-load oxygen at the tissue level. The possibility also exists that bilirubin may bind to haemoglobin.

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† Research project No. 36.5.47 approved by the Faculty Ethics and Research Committees.

# WATER AND ELECTROLYTE HOMEOSTASIS IN SHEEP WITHOUT FUNCTIONAL COLONS[†]

*R.A. Meintjes & H. Engelbrecht*

Department of Physiology

The mammalian colon has been likened to the distal part of the nephron tubule with respect to sodium and water homeostasis. In this region of the digestive tract, as in the collecting duct, the degree to which sodium and water are reabsorbed is variable and depends on the animal's requirements, i.e. reabsorption is facultative. In this investigation, these aspects of kidney and colon function were compared using sheep with ileorectal anastomosis (IRA sheep) as experimental models.

The sheep were individually housed in metabolic crates throughout the trial. Drinking water was freely available to both control and IRA sheep during Phase 1. During Phase 2, the IRA sheep were limited in their water intake to that of the controls in Phase 1. Throughout both phases, each animal received 10g NaCl per day via the drinking water.

Water intake, loss of sodium, potassium and water via the faeces and urine were monitored. Variables such as glomerular filtration rate (taken as being equal to the endogenous creatinine clearance), the fractional excretions of electrolytes and electrolyte free water reabsorption were calculated. Plasma aldosterone concentrations in control group and IRA group on *ad libitum* and restricted water intake were compared.

Electrolyte loss via the faeces was much higher in IRA sheep than in the control animals. During Phase 1, IRA sheep drank about 50% more water, while losing more water via the faeces and the urine, than the control group. Loss of electrolyte free water via the kidneys was sustained by the IRA sheep and renal retention of water was entirely secondary to the high degree of sodium reabsorption in these animals (fractional excretion of sodium of IRA sheep = 0,29%; of control sheep = 1,68%). During Phase 2, the urine volume of IRA sheep declined below that of the control animals (on similar water intake); fractional excretion of sodium was further reduced to 0,08% and electrolyte free water was retained. During both phases, plasma aldosterone concentrations were markedly higher and plasma potassium concentrations lower in IRA sheep than in control sheep.

In conclusion, the role of the colon was quantitatively described for water and electrolyte balance in sheep. Following ileorectal anastomosis, animals supplemented with at least 10g NaCl per day are able to maintain physiological balance by relatively major changes in kidney function, some of which are potentiated by the effects of aldosterone.

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[†] Research project No. 36.5.33 approved by the Faculty Ethics and Research Committees.

# EFFECT OF WATER BALANCE ON NITROGEN RETENTION IN SHEEP†

*J.G. van der Walt, E.A. Boomker, R.A. Meintjes, W.A. Schultheiss[1], M. Grobler & H. Engelbrecht*

Department of Physiology, [1] Department of Veterinary Ethology

Large parts of Southern Africa are characterized by grassland regularly subjected to long periods of low rainfall. Many grasses store protein in their roots during the dry period of the year. As a result, grazing herbivores often have to contend with a protein content (crude protein or CP) of less than 3%. The grazing ruminant may remain in a positive nitrogen balance at CP levels above 6%, in contrast to most monogastric mammals which require about 12% CP or more. This is achieved by recycling urea. Urea, however, is also excreted by the kidney, where it plays an important role in the creation of the osmotic gradient between the cortex and the medulla. We therefore examined the effect of restricting water intake in sheep fed either a control (CP=7%) or a high nitrogen (CP=12%) diet.

A group of 16 SA Mutton Merino wethers were randomly allocated to 2 groups, one of which was fed a diet based on chaffed wheat straw (CP = 7%), while the other group received a similar diet supplemented with urea (CP = 12%). After 2 weeks of adaptation to the diet, 4 sheep from each group were placed into metabolism crates and allowed a further week to adapt to their surroundings. Half of each group were then restricted to half their previous *ad libitum* water intake for 7 days. During the last 4 days of this period, water and feed intakes and faeces and urine outputs were measured, and samples taken for analysis. Feed, faeces and urine samples were analysed for, *inter alia*, total nitrogen (from which CP may be calculated), urea, ammonia and organic matter. The remaining sheep were similarly treated so that every sheep within each group was subjected to both an *ad libitum* and a restricted water regimen. The groups were not crossed over with respect to diet.

The group fed the control diet consumed 783±87, 47,2±5,3 and 2800 g/day organic matter (OM), CP and water, respectively, when allowed free access to water. Restricting water intake to about 1400 g/day reduced the intake of OM and CP to 608 and 36,6 g/day respectively. In the case of the group fed the high nitrogen diet, reducing the water intake from 2800 to 1400 g/day reduced the OM and CP intakes from 936±64 and 103,4±7,1 to 654±148 and 72,3±16,4 g/day, respectively. As a result, urine output decreased from 945±326 and 772±150 ml/day to 500±59 and 509±276 ml/day for sheep fed the control or high nitrogen diet, respectively. Water restriction in the high nitrogen group decreased the amount of nitrogen retained from 39 to 12 g/day, largely as a result of the decreased intake of nitrogen (about 30 g/day). Although the intake of OM also decreased proportionately, the amount lost in the faeces remained the same, suggesting that OM digestibility was decreased (from 68 to 52%). In the case of the group fed the control diet, restricting the water intake improved nitrogen retention from 1,1 to 6,4 g/day, despite the depressed intake of feed CP. There was also an increase in OM digestibility (53 to 66%).

While a restricted intake of water may adversely affect sheep fed a diet containing adequate amounts of CP, the same treatment would appear to be advantageous to sheep fed a diet close to the minimum requirement.

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† Research project No. 36.5.48 approved by the Faculty Ethics and Research Committees.

# SURVEY OF THE DESIGNS OF RACEHORSE STABLES IN THE PRETORIA, WITWATERSRAND AND VEREENIGING AREA OF SOUTH AFRICA[†]

*R.J. Lund, A.J. Guthrie & V.M. Killeen*

Equine Research Centre

The aim of this study was to survey current designs of and management practices at racehorse stables in the Pretoria, Witwatersrand and Vereeniging (PWV) area of South Africa.

The stable yards included in the survey were racehorse training yards in the PWV area housing more than twenty horses in training. Twenty-four yards, with a total of 228 buildings of both open-fronted and barn-style stables were surveyed. A total of 4765 stables were included in the survey. The size and occupancy of each building was recorded. Measurements included dimensions of each box and the size of the ventilation openings. Management practices, including the type of bedding and the feed handling procedures used, were also recorded. Predicted ventilation rates were calculated with the aid of custom computer programs.

Most racehorses were kept in loose boxes, bedded on straw or sawdust and remained stabled while the stable was cleaned. The average floor area per animal was 13 $m^2$ and the airspace per animal was 55 $m^3$. The floor averaged heat transfer coefficient ranged from 2 to 14 $W/m^2/K$. The predicted air change rate by natural convection in calm winds was 7,0 air changes per hour when all vents were open, but this was reduced to 2,2 when the doors and shutters were closed. The physical dimensions of the stables included in this survey were very similar to those of stables in other countries. The floor averaged heat transfer coefficient of the stables was markedly higher than those reported overseas. In the present survey, approximately 25% of all horses were kept in buildings in which the maximum predicted air change rates were less than 4/h when all vents were open. Upon closing all variable vents, the predicted air change rate was less than 4/h in 35% of the stables surveyed. These findings show that a large proportion of the racing stables in use in the PWV area are ventilated at a rate that is less than the recommended minimum value, and that in excess of 80% of all the stables were always ventilated at a rate below the recommended value of 8 air changes per hour.

While the dimensions of stables included in this survey are similar to those used overseas, the local buildings are poorly insulated. The poor insulation and lack of permanent vents in the buildings almost invariably resulted in suboptimal building ventilation. This, in turn, resulted in poor air quality, which may predispose the horses occupying the buildings to respiratory diseases.

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[†] Research project No. 36.5.24 approved by the Faculty Ethics and Research Committees.

# A PROSPECTIVE STUDY COMPARING THREE METHODS OF STABILISING THE STIFLE JOINT FOLLOWING CRANIAL CRUCIATE LIGAMENT RUPTURE IN DOGS[†]

*G.L. Coetzee*

Department of Surgery

Rupture of the cranial cruciate ligament is a very common orthopaedic problem in the dog. A wide variety of techniques for surgical repair have been described; however, it remains unclear how the various techniques compare in performance. The intra-articular "under-and-over" technique, using autogenous fascia lata (n = 20), was compared with a combination of intra- and extracapsular "under-and-over-the-top" (UOTT) fascial reconstruction (n = 49) and with an extracapsular orthopaedic wire loop stabilisation method (n = 29). This study was undertaken in medium and large-breed dogs (> 15 kg) presented with unilateral ruptured cranial cruciate ligament. Case selection was done at random, with the exception of the "under-and-over" technique, which was not used on medium breed dogs, because of the size of the implant. Functional limb usage of the operated limbs was evaluated according to a clinical grading system within the first 6 weeks, and again at 12 and 24 weeks.

Dogs treated with the intra-articular "under-and-over" technique started partial weight-bearing on the operated limbs within the first 3 to 5 weeks. Return to full function with no or only occasional lameness was recorded in 17/20 (85%) of the dogs treated with this technique after 24 weeks. Those treated with the UOTT technique returned to partial weight-bearing within 4 to 6 weeks, and exhibited slightly more cranial drawer movement 6 and 12 weeks after surgery. During this medium-term follow-up period, the results in 40/49 (81%) of the dogs treated with the UOTT method, were comparable with the "under-and-over" method. Distinctive radiological differences could not be detected between the two fascial substitute techniques 24 weeks postoperatively. The majority of dogs with the extracapsular wire loop responded very well initially and used the operated limbs within the first 2 weeks; however, their functional limb usage deteriorated after 6 weeks. All the wires broke within the first 12 weeks after placement. Most of the 17/29 dogs (59%) with the extracapsular wires that exhibited functional limb use without lameness after 24 weeks, belonged to the medium size breeds. Six of the 29 dogs in this group (20,7%) needed replacement of the wires between the first 6 and 12 weeks. These specific dogs had advanced osteoarthrosis 24 weeks postoperatively.

The results of the intra-articular "under-and-over" technique in large-breed dogs are in agreement with other studies. The UOTT fascial reconstruction technique was found to be more reliable especially in large size dogs, compared to the extracapsular wire loop stabilisation method.

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[†] Research project No. 36.5.35 approved by the Faculty Ethics and Research Committees.

# PEDICULECTOMY FOR THORACOLUMBAR SPINAL DECOMPRESSION IN THE DACHSHUND

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Pediculectomy is defined as the surgical removal of the lateral bony wall of the spinal canal to provide direct access to the epidural space ventrally and laterally to the spinal cord. This approach allows good visualisation and atraumatic surgical removal of intervertebral disc protrusions and extrusions.

A prospective study of thirty-two clinical cases of disc disease in the Dachshund was undertaken to correlate the clinical presentation and myelographic changes with the surgical findings and outcome. Based on the findings of neurological examinations four prognostic categories (good, fair, poor and very poor) were defined. The diagnosis was confirmed and the exact localization of the lesion determined by lumbar myelography. The standard dorsal approach to the spine was used. The pedicle at the site of the lesion was partially or totally resected using high speed burring. The prolapsed disc material was carefully removed and a discectomy of adjacent disc spaces was performed.

The cases with a good prognosis showed a speedy recovery after decompression. The cases with a fair or poor prognosis recovered but some proprioceptive deficit remained. One case with a very poor prognosis recovered, while four cases had to be humanely killed.

It is important to remove extruding disc material from the spinal canal. The ability to accurately localise the compressive lesion makes it possible to use the limited approach of the pediculectomy technique to achieve this. This technique is less invasive and traumatic than other described techniques. The results obtained in this study, using the pediculectomy technique, compare favourably with reported results using more invasive techniques.

# THE USE OF COMPUTER-AIDED IMAGE ANALYSIS FOR THE OBJECTIVE EVALUATION OF EQUINE ENDOMETRIAL BIOPSIES[†]

*C. Gerstenberg & D.H. Volkmann*

Department of Theriogenology

Due to operator subjectivity, the histopathological evaluation of equine endometrial biopsies yields inadequately reliable results for research work. The aim of this project was to write a computer programme which would guide the operator through the systematic evaluation and data processing of equine endometrial histopathology by means of a more objective image analysis system.

A single endometrial biopsy was used to develop a computer aided image analysis programme for the Contron Image Analyzer, primarily intended for more simplistic industrial applications.

Light microscopic images of plastic embedded, 2 $\mu$m thick and 20 mm long haematoxylin and eosin (H&E) stadined sections were converted into black and white enhanced images with the image analyzer and then evaluated. The programme developed for evaluation consists of 3 parts: Part 1 evaluates gross ($\geq$ 2800 $\mu m^2$) lesions which are then excluded from subsequent measurements; Part 2 evaluates the features of the luminal epithelium and stratum compactum and Part 3 evaluates the features of the stratum spongiosum.

General histological features and changes due to inflammation, fibrosis, necrosis as well as haemodynamic changes and glandular pathology are measured. Broadly stated, within each part of the programme epithelial height (luminal and glandular epithelium), number of cells per unit surface area, differential cell counts (fibroblasts, neutrophils, lymphocytes, plasma cells, eosinophils, macrophages and mast cells) as well as the number and size of blood vessels and lymphatics are quantified. Density, nesting, branching, dilation, and activity of glands are also quantified.

The programme is designed in such a way that it prompts the operator to perform each step of the evaluation in a systematic manner, repeating all observations on 5-7 microscopic fields.

The programme automatically extracts the data pertaining to each biopsy into data files in a format which allows for easy further processing by any statistics programme. The latter includes the calculation of standard statistics for repeated observations and of histologically meaningful variables, such as density of cell types in different endometrial strata, ratios between cell types, number of gland cross section per unit surface area and many more.

The programme will now be applied to compare the findings between conventional and image analysis evaluations of equine endometrial biopsies and to establish the repeatability of variables for mares of different fertility status and at various stages of the reproductive cycle. If the programme proves sufficiently discriminatory this study can provide the basis for countless other applications in histology and histopathology.

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[†] Research project No. 36.5.55 approved by the Faculty Ethics and Research Committees.

# RESEARCH PROGRAMME/NAVORSINGSPROGRAM

## Plakkate/Posters

### ELECTRON MICROSCOPIC STUDY OF BUFFALO (*Syncerus caffer*) SPERMATOZOA

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The structure of African buffalo spermatozoa has not been studied by electron microscopy. The aim of this study was to describe the ultrastructure of normal buffalo spermatozoa in order to obtain base line data required for future reference. This information may enable us to compare normal sperm with those of animals subjected to toxic substances or other stress factors in their environment. In this way spermatozoa could provide an important test method for toxicity.

Samples of caudal epididymal spermatozoa were collected in the Kruger National Park from ten healthy, sexually mature buffalo bulls during a routine culling operation carried out by officials of the National Parks Board of South Africa. Specimens were prepared for both SEM and TEM using standard methods.

SEM demonstrated that the spermatozoon consisted of a dorsoventrally flattened, paddle-shaped head and a tail. The apical tip of the head displayed a distinct, unilateral acrosomal bulge. Fine filaments projected from the midpiece and principal-piece of the tail, and stalk-like appendages occurred on some cytoplasmic droplets. Sections of spermatozoa revealed typical mammalian features. However, the mitochondria of the pars ascendens were coaxially arranged, and were positioned deep within the neck region. A cone-shaped annular ring of dense material demarcated the midpiece and principal-piece. A retro-annular recess typical of mammalian spermatozoa was absent. The fibrous sheath of the principal-piece extended between the distal portion of the annulus and the axoneme to form a close contact with the base of the annular ring.

Electron microscopy demonstrated the basic ultrastructure and surface morphology of buffalo spermatozoa and indicated that they have the same general structure as that of other mammals. However, there appear to be differences concerning the extent of the pars ascendens in the neck region, the absence of a slender neck region and in the structure of the annulus.

# MONITORING THE NUTRITIONAL STATUS OF THE IMPALA (*Aepyceros melampus*) AND AFRICAN BUFFALO (*Syncerus caffer*)†

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Several authors have reported on the feasability of using faecal indices to monitor herbivore nutritional status. The results proved promising and, for this reason, it was decided to use faecal nitrogen (FN) and phosphorous (FP) in a regular monitoring system for impala and buffalo in the Kruger National Park (KNP). The purpose of this ongoing study is to determine at what stage, and in which areas, these species become nutritionally stressed. This could have important implications in understanding reproduction and survival rates as well as implementing pro-active management measures for controlling stocking density for the species concerned.

Only visibly wet (fresh) faeces were collected and care was taken not to include any sand and/or plant material. Each sample was identified according to species, date, area of collection and collector. No significant inter-animal or inter-collector differences were found in any particular area, and thus samples were pooled. From 7 landscape areas in the KNP, 126 pooled samples from impala and 35 from buffalo, each representing more than 5 animals, were analysed between October 1991 and October 1992, a period during which the KNP experienced one of its most severe droughts. Light spectrophotometry and Kjeldahl methods were used to analyse FP and FN respectively.

Results from impala showed that there were significant differences in both FP and FN between months, and between different landscapes ($p < 0,05$). Analysis of variance showed that 63% and 86% of the variation in FN and FP, respectively, was explained by the influence of month of year and landscape type. Results from buffalo indicated that there were significant differences in both FP and FN between months, and only in FP between landscapes. In buffalo, 84 and 48% of the total variance in FN and FP, respectively, was explained by the influences of month and landscape type. In both species, FP values on basalt were consistently higher than on granite, whereas FN values were higher on granite than on basalt due to utilization of available browse. These findings may have been partly due to higher $Ca^{2+}$ intake on basalt and higher tannin intake on granite. The inverse relationship between FP and FN on basalt may be explained by a possible decrease in dietary nitrogen due to poor veld quality which may cause a decrease in diet digestibility leading to a rise in FP. These results show; 1) FN and FP should never be evaluated separately, 2) further work is needed on the effect of tannins, 3) the impala is an extremely adaptable animal when it comes to diet selection during droughts, and 4) dietary protein is limiting for buffalo on both granite and basalt as is evident from their high mortality rate during the past drought.

† Research project No. 36.5.16 approved by the Faculty Ethics and Research Committees.

# THE USE OF A WATER SOLUBLE PLASTIC RESIN TO PREPARE LARGE HISTOLOGICAL SECTIONS OF ENDOMETRIAL BIOPSIES FOR COMPUTER-AIDED IMAGE ANALYSIS†

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Computer-aided image analysis is a novel method to objectively analyze histological sections. Plastic resins are known as superior embedding media for cutting ultrathin sections of very small tissue specimens for electron-microscopy. The aim of this project was to develop a method of processing and embedding equine endometrial biopsies in a resin which would adequately preserve histological detail, allow for cutting of 20 mm long sections and permit suitable staining to achieve an image resolution and contrast necessary for image analysis.

Endometrial specimens were taken with a Yeoman biopsy forceps, carefully flattened on a piece of paper and then immediately fixed in 10 % phosphate buffered formalin. During processing the tissue was first dehydrated by moving it through a sequence of 6 increasing percentages (50 - 96 %) of alcohol for 30 min each. After being left in the resin mixture (Bio-Rad Plastic Embedding Medium, Premier Technology) overnight to be impregnated, the specimens were placed into a 5 mm wide flexible mould and held straight and upright while the resin mixed with the hardener was poured in and started to polymerise. An aluminium holder for the microtome was then placed on top. In order to harden the block properly it was placed into a vacuum chamber (48-103 kPa) for 12-24 h. An automatic microtome (Anglia Scientific, AS 500) was used to cut the plastic embedded specimens with 0.6 mm glassknives. Straight sections of 2 $\mu$m thickness were cut at right angles to the mucosal surface across the whole length of the biopsy (20 mm) and routinely transferred onto microscope slides.

The sections were stained with special haematoxylin and eosin stains suitable for the embedding medium (30 min haematoxylin, 10 s eosin). Toluidine blue (1 %) staining for a few seconds as used for electron-microscopy was used on duplicate sections to highlight mast cells. The sections were routinely mounted.

Processing and embedding endometrial biopsies by the method described above resulted in optimal preservation of histological detail. Automatic sectioning with glassknives perpendicular to the endometrial mucosa made it possible to obtain straight, thin sections which covered the whole length of the biopsy (20 mm). Sections of 2 $\mu$m thickness were found to be ideal for image analysis, ensuring that the nuclei were individually recognisable but not cut into fragments too small for reliable identification. Staining with H&E ensured a level of contrast and resolution adequate for image analysis.

Preparing specimens in this way makes image analysis a feasible option for evaluating equine endometrial biopsies. Water soluble plastic resins thus have countless further applications when fairly large histological specimens need to be prepared for detailed evaluation.

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† Research project No. 36.5.55 approved by the Faculty Ethics and Research Committees.

# A COMPARISON OF NEMATODE PARASITES OF HORSES FROM TWO MANAGEMENT SCHEMES†

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Internal parasites of horses, comprised mainly of nematodes, are one of the causes of colic. Little information exists concerning the presence of these parasites in horses in South Africa. No information is available which compares horses housed under differing management schemes. The aim of the present study was to compare the internal parasites of two groups of horses housed at the Faculty which had been managed differently. Group 1, chiefly ponies, comprised cycling or early pregnancy mares. They were maintained in paddocks, given supplemental feed and treated four times a year with antiparasitic remedies. Group 2 horses, chiefly Thoroughbreds, grazed daily on irrigated pastures and received antiparasitic remedies twice a year.

The two groups were each divided into conventional and strategic subgroups. The conventional subgroups were treated as they had been in previous years. The strategic subgroups were treated with an anthelmintic if the nematode egg count was larger or equal to 300 eggs per gram of faeces.

Monthly faecal analyses were carried out which included nematode egg counts for each horse and larval cultures for each subgroup. The nematode eggs which were recovered included those of strongyles, and *Parascaris equorum* and *Strongyloides*. Differentiation of third-stage larvae ($L_3$) from cultures distinguished between small strongyles (cyathostome) and large strongyles. Statistical analyses were performed on the total mean nematode egg counts for conventional and strategic subgroups within each group of horses.

Differences between subgroups were significant for Group 1 but not for Group 2. A considerable financial saving was evident, however, for the strategic subgroup as compared to the conventional, particularly in Group 1. That a resistance to the antiparasitic remedies did not develope is a further advantage. A strategic programme of control can also be developed, with basic management that includes monitoring of internal parasite levels through faecal examination.

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† Research project No. 36.5.21 approved by the Faculty Ethics and Research Committees.

# THE QUALITY OF FRESHLY COLLECTED CANINE SEMEN AND ITS FREEZABILITY†

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Department of Theriogenology

The freezability of an ejaculate may be expressed in terms of progressive motility and acrosome integrity post-thaw. Maintenance of these factors over time is also important. Canine ejaculates vary with respect to both their quality immediately after collection and their freezability. The aim of this study was to determine whether these evaluation variables of freshly collected canine semen are significantly correlated with freezability.

The semen of 5 dogs was used. The quality of semen freshly collected from all donors was acceptable, according to routinely used criteria. The sperm-rich fractions of a total of 73 ejaculates (6 - 18 ejaculates per dog) were evaluated using standard procedures for sperm concentration, percentage progressive motility and sperm morphology, immediately after collection and post-thaw. Motility was evaluated using a phase-contrast microscope with a hot stage set at 37 °C. Sperm concentration was determined in a haemocytometer. Smears for the evaluation of sperm morphology were prepared at 37 °C and stained with Eosin Nigrosin, after which they were mounted under Entellan mounting medium. Sperm morphology was determined at 1000x magnification (under oil). All evaluations were performed by one person. All ejaculates were frozen according to the same method, after extension in Triladyl with 0,5% Equex STM paste. Pairwise linear correlation coefficients were calculated between variables of fresh and post-thaw semen.

Progressive motility post-thaw and maintenance of motility were most strongly and significantly correlated with the following variables of freshly collected semen: Incidence of distal droplets, nuclear vacuoles, major head defects ($r < -0,5$, $p < 0,0001$) and microcephalic sperm ($r < -0,4$ and $p < 0,01$). Percentage sperm with abnormal acrosomes and maintenance of acrosome integrity were both most strongly and significantly correlated with the incidence of Dag defects in fresh semen ($r = 0,46$, $p < 0,001$; $r = -0,45$ and $p = 0,01$, respectively). Maintenance of acrosome integrity was also strongly and significantly correlated with the incidence of damaged acrosomes and double forms in fresh semen ($r = 0,46$, $p < 0,001$; $r = 0,4$ and $p < 0,01$, respectively). Progressive motility of fresh semen was significantly correlated with maintenance of motility and maintenance of acrosome integrity ($r = -0,39$, $p < 0,01$; $r = 0,3$ and $p < 0,05$, respectively). Neither the percentage of sperm with normal morphology, nor sperm concentration, were significantly correlated with any of the criteria for freezability ($p \geq 0,19$).

It may be concluded that, within the range of semen quality found in this sample, the freezability of a canine ejaculate depends upon the quality of the freshly collected semen, with the exception of the concentration of sperm in the sperm rich fraction and the percentage sperm with normal morphology.

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† Research project No. 36.5.61 approved by the Faculty Ethics and Research Committees.

# THE ULTRASTRUCTURE OF RETAINED CYTOPLASMIC DROPLETS IN OSTRICH SPERMATOZOA

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The mammalian cytoplasmic droplet represents a small amount of excess cytoplasm not removed from the late stage spermatid as a residual body during spermiation. This droplet is generally shed during sperm maturation in the epididymis but its retention in ejaculated sperm is considered to be an abnormality. Although the ultrastructure of the mammalian cytoplasmic droplet has been extensively studied little information is available on birds. This paper describes the fine structure of the cytoplasmic droplet of ostrich sperm.

Semen samples collected from five mature male ostriches during the breeding season were fixed overnight in 2% glutaraldehyde in Millonig's phosphate buffer. The samples were post-fixed in similarly buffered 1% osmium tetroxide and prepared for TEM using standard techniques. Thin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips 301 TEM operated at 80kV. Glutaraldehyde-fixed sperm suspended and diluted in Millonig's phosphate buffer were layered onto poly-L-lysine-coated mica sheets and prepared for SEM using standard techniques. The samples were viewed in a Philips XL 20 scanning electron microscope operated at 10kV.

Retained cytoplasmic droplets were observed on sperm from all five birds but the incidence was variable. The droplets were almost exclusively restricted in location to the neck region of the sperm cells, overlapping both the base of the head and the proximal section of the midpiece. They were eccentrically positioned and with SEM presented a follicular appearance. A few droplets were associated with the sperm head and the midpiece/principal-piece junction of the tail. Tail bending in the region of the midpiece was associated with large cytoplasmic droplets. TEM revealed that the cytoplasmic droplet was bounded by the plasmalemma of the sperm cell. The droplets were composed of a moderately dense, granular ground substance containing pale lipid-like droplets, vesicles, vacuoles, membranous structures, and occasional mitochondria. The latter were similar in structure to those surrounding the midpiece region of the tail. The superficially situated lipid-like droplets and vesicles often caused localised bulging of the overlying plasmalemma.

The positioning of the cytoplasmic droplet in the neck region of ejaculated ostrich sperm is similar to that reported in mammals and is viewed as a major abnormality. This observation may also indicate that the juncture of the head and neck is the position where excess spermatid cytoplasm is removed as a residual body during spermiation in the ostrich. No clear evidence was obtained that the cytoplasmic droplet of ostrich sperm undergoes a distal migration as occurs in mammals. Ostrich cytoplasmic droplets differ from those of mammals in respect of their content of lipid-like droplets and paucity of membranous structures but are similar in most respects to those of the painted turtle. This similarity re-emphasises the close relationship that exists between ostrich and Chelonian sperm.

# RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN CHEETAHS[†]

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Department of Veterinary Tropical Diseases

As a result of findings of an extensive genetic and physiological analysis, previous workers have proposed that the cheetah shows monomorphism at the major histocompatibility complex (MHC). This has led to a proposal that the cheetah suffers from an immunodeficiency syndrome and is highly susceptible to disease. Previous work performed in our laboratory has shown that the humoral and cell mediated immune functions of cheetahs are intact.

Heparinised blood samples were collected from 15 healthy captive cheetahs into evacuated blood collection tubes. Nuclear DNA was harvested from leucocytes using standard techniques. Restriction fragment length polymorphism patterns of the MHC I and MHC II genes were examined using Pst 1 and Bam H1 restriction enzymes and human DNA probes to the MHC I and MHC II genes.

Specimens from all 15 animals were repeatedly hybridised. Hybridisation was successful for samples from no more than three animals in each batch. The poor repeatability of the hybridisation is probably due to lack of specificity of the human probes for cheetah DNA. By pooling the results from all of the hybridisation assays, it became apparent that the cheetah is restricted at the MHC I locus but not at the MHC II locus.

The MHC I locus contains genes controlling graft rejection, whereas the MHC II locus contains the genes for immune response. The results of the present study show that cheetahs are not immunodeficient, as the immune system is capable of responding to foreign antigens, but they are immunotolerant towards antigens from other cheetahs.

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[†] Research project No. 36.5.8 approved by the Faculty Ethics and Research Committees.

# FELINE IMMUNODEFICIENCY VIRUS IN NON-DOMESTIC FELIDS IN SOUTHERN AFRICA

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Feline immunodeficiency virus occurs commonly in domestic cats world-wide. It has also been found in non-domestic felids in zoos in America and Europe. Its occurrence in captive and free-living non-domestic felids has not been investigated previously in southern Africa.

Sera were obtained from free-living non-domestic felids from the Kruger National Park, Etosha National Park, Umfolozi Game Reserve, Botswana, Namibia, and from captive non-domestic felids in the Pretoria and Johannesburg Zoos. These sera were examined for the presence of feline immunodeficiency virus (FIV) antibodies using a commercially available ELISA kit (IDEXX, Portland, Maine); an ELISA that was developed in-house using a genetically engineered antigen representing the p24 structural protein of the virus; and Western Blot analysis.

FIV antibodies were detected in a number of lions and two leopards from the Kruger National Park, one leopard from Botswana and captive lions from the Johannesburg and Pretoria zoos. The captive lions at both zoos originated from the Kruger National Park. None of the animals from Namibia or Natal were positive.

These results show that non-domestic felids in the Kruger National Park and Botswana have been exposed to FIV. The clinical importance of these findings is still under investigation.

# CASE REPORT: RUMEN FUNCTION FAILURE DESPITE AVAILABILITY OF ADEQUATE SUPPLEMENTARY FEED.

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Severe droughts are a normal phenomenon on the African continent. Pro-active destocking is a necessary measure to prevent livestock mortalities. The remaining animals may then be fed at: 1) sub-maintenance levels whereby a calculated drop in body mass is to be expected, 2) maintenance levels or 3) in selected animals at desired production levels.

At the end of October 1992, a farm in the drought-stricken Tolwe district of the north western Transvaal was visited. Approximately 200 cows were found to be in a very poor body condition (average score 1 on a scale of 1-5) with some showing signs of anaemia, "bottlejaw", rumenstasis with impaction and loose, fetid, brown faeces. The animals had access to good quality pea hay (total crude protein = 15,5% and acid detergent fibre = 33,5%) and a protein-energy-phosphorous lick all of which had only been introduced 10 days earlier. Prior to this sudden change in diet, the herd had been picking up dry tomato plant harvest residues, some maize stover, a phosphate-salt lick and begasse. It was evident that there had not been sufficient dry matter available to the animals.

Clinical examination of several cows revealed that the rumen pH values were in the high range (about 7,5; normal = 6,8), and the rumen contents exhibited very extended methylene blue reduction times. Chemical pathology revealed a hypo-albuminaemia and a hyperglobulinaemia. Both the haematocrit and the serum urea nitrogen levels were at the lower range of normal. Exploratory laparotomy and rumenotomy were performed on two animals. Both showed a hydroperitoneum and the rumen was filled to capacity with long, undigested, fetid, dry pea hay. Several short strands of non-penetrating wire and large amounts of sand were found in the forestomachs.

A low daily protein intake prior to the availability of good quality pea hay was not sufficient for the normal cellulolytic rumen flora to survive. Even the low-protein bagasse could not sufficiently improve fibre digestion. Hunger prompted the animals to ingest large quantities of the pea hay, leading to rumen impaction. This induced rumen stasis, resulted in failure to clear the cardia, thereby inhibiting normal emptying through the reticulo-omasal opening. Rumination did not take place and rumen retention time increased, leading to a rumen alkalosis with the eventual decomposition of the contents. Timely supplementation with sufficient protein for microbial breakdown of poor quality roughages, so often the only roughages available during droughts, could have prevented unnecessary mortalities.

# CHANGES IN BLOOD CLOTTING, BLOOD CALCIUM, BLOOD PROTEIN, HAEMATOCRIT AND WHITE CELL COUNTS IN SHEEP WITH EXPERIMENTALLY-INDUCED HEARTWATER

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Department of Medicine

Heartwater was induced in seven healthy adult Merino sheep by inoculating each of them intravenously with a single dose (5 mℓ of infected blood) of the "Welgevonden" stock of *Cowdria ruminantium*. The rectal temperature of each animal was recorded twice daily. Clinical disease was considered to have commenced when the morning temperature reached 40°C. Rectal temperatures above 40°C were recorded in all 7 sheep from 8-15 days post infection. Blood was collected for haematology and clinical chemistry on days -13, -6, -5 and -1 from infection on the day of infection and then daily from Day 7-13 post infection. On day 13 two samples were collected several hours apart from each of 4 sheep. One sheep died on Day 12, five died or were euthanased on Day 13 due to severe clinical signs and one was euthanased on Day 15.

Results showed a marked decline in thrombocyte count during the acute stage of the disease. This was associated with significant increases in both prothrombin time (PT) and activated partial thromboplastin time (APTT). Fibrinogen increased whilst there was no detectable increase in fibrinogen degradation products (FDP). At the same time that total serum protein (TSP), albumin and globulin declined very sharply, total calcium showed a progressive fall, while ionised calcium rose initially and then declined. The total leucocyte count increased terminally, whilst the haematocrit declined progressively.

The observed changes in blood clotting warranted a laboratory diagnosis of a consumption coagulopathy, e.g. DIC. Although macropathological changes (haemorrhages and oedema) associated with DIC are often observed in cases of heartwater, the presence of micro-thrombi have only been described once before. The high pathogenicity of the *Cowdria* stock used in this experiment may also have played a role in the pathogenesis of the observed DIC.

The decline in blood protein concentration again confirms the severity of the microvascular permeability defect which develops in this disease. The reason for the increase in ionised calcium during the acute stage of the disease is not immediately apparent. The role that the terminal fall in ionised calcium concentration plays in the death of the animal needs to be investigated, as does the pathogenesis of the normocytic, normochromic anaemia which develops during the course of the disease. The exact reason for the terminal leucocytosis is also not clear.

# DARK, FIRM AND DRY SYNDROME IN FEEDLOT CATTLE[†]

*J.G. van der Walt, H. Krogmann[1], J.H. Taylor, J. van Rensburg, M. Grobler & N. Engelbrecht*

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The dark, firm and dry (DFD) syndrome causes large losses to the cattle feedlot industry. This syndrome is caused by a loss of muscle glycogen immediately prior to slaughter, which lowers muscle lactic acid concentration. Stress-related factors affect the rate of glycogenolysis, as shown by the increased incidence of DFD in cattle prone to mounting behaviour. The apparent variation in the incidence of DFD in South Africa between feedlots prompted this investigation.

While feedlots B and C used mixed breed cattle (*inter alia* Hereford, Afrikaner and Brahman), the cattle from Feedlot A were purebred Fries. At feedlots A and C, trenbolone acetate (140 mg) was implanted in 9 month old cattle, while at feedlot B a preparation of oestradiol benzoate plus progesterone (20 and 90 mg respectively, at 6 weeks) and trenbolone acetate (140 mg, at 4 months) was implanted. Groups of 20 cattle were randomly selected at each feedlot. Blood samples and muscle biopsies were taken at the feedlot, after arrival at the abattoir, and after slaughter. Values were obtained for haematocrit, plasma free fatty acid and glucose concentrations and creatine kinase activity. The muscle samples were analyzed for pH. The carcasses were graded for colour (values of 7 or 8 were classified as DFD).

Carcasses graded as DFD amounted to 3, 7, and 7 at feedlots A, B and C respectively. The colour correlated well with the $[H^+]$. If the threshold is set at $pH=5.8$, then only 2 of the DFD carcasses ($n=17$) displayed values lower than this. Furthermore, 5 of the carcasses that were acceptable ($n=43$) displayed pH values higher than this. Of all the variables investigated, only plasma free fatty acid concentrations in cattle still on the feedlot correlated with either colour or pH. The concentrations of FFA in plasma of cattle taken after arrival at the abattoir or immediately after slaughter showed no correlation with either colour or pH. Although the number of suspect carcasses were identical at feedlots B and C, there were no carcasses at feedlot B that graded below 6.

Depletion of muscle glycogen stores prior to slaughter raises the pH of the meat. As a result, the colour of the meat grades at an unacceptable 7 or 8. Despite similar conditions at all the feedlots, the lower incidence of DFD from feedlot A is probably related to the placid nature of Fries cattle. The correlation between FFA concentrations in cattle still on the feedlot and their eventual grading after slaughter, may point to an increased sensitivity to acute stress.

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[†] Research project No. 36.5.81 approved by the Faculty Ethics and Research Committees.

# THE POSITIVE INFLUENCE OF HORSE-RIDING ON A CEREBRAL PALSY CHILD

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A normal child who survived a hypoxia episode post-operatively at the age of three, was as a result quadriplegic. He displayed no purposeful arm movements and the middle finger was in a flexodominant position. His legs were extremely extensor spastic, dystonic with moderate adduction and ankle plantar flexion. He was mainly dysphoric, with a low frustration tolerance. He was not able to speak, but could see and hear.

A Shetland pony gelding was used to apply riding therapy from August 1992 (date of accident) for a period of 6 months. Field research was carried out over a period of one year, during which the child rode daily for half an hour. The child has subsequently been evaluated by neurologists, pediatricians, physiotherapists, occupational therapists and an orthopaedic surgeon.

The positive influence of the treatment appears to be remarkable, as the boy could relax more easily and the control of his head and rump improved. His perception of a midline returned. As a result of the more relaxed muscles, the other physiotherapy given was more successful. His quality of life has improved, as shown by a greater degree of relaxation and a degree of speech encompassing a vocabulary of 15 words. After 5 months he was able to use his arms to point out directions and was able to use his hands, albeit with difficulty.

The explanation for the positive influence of riding may include:

1. The soft rocking movement of the horse may have influenced the vestibular system resulting in muscle tone normalisation.
2. The child sat in a reflex inhibiting position on the horse, namely hips in flexion and abduction.
3. After overcoming an original fear of the unstable surface of the saddle, the physical relaxation the motion has provided has given the child more self confidence.
4. The warmth of the pony's body could have stimulated muscle relaxation.
5. The movement of horse and child over uneven surfaces resulted in postural adaptations, especially in relation to head and rump.
6. The frustration of immobility is overcome thereby opening up a new world to the child.

# THE DETECTION OF BLUETONGUE VIRUS NUCLEIC ACID USING THE PCR AND *IN SITU* HYBRIDIZATION TECHNIQUES

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The current laboratory diagnosis of bluetongue is cumbersome and can take 14 days or longer. This has led to the investigation of other rapid and sensitive techniques for the detection of the virus. The polymerase chain reaction (PCR) and *in situ* hybridization (ISH) technique have been widely used for the rapid and specific detection of viruses. In this study, we evaluated PCR for the detection of bluetongue virus nucleic acid in infected cell cultures, and ISH in tissue sections.

The sensitivity of PCR to detect BTV in infected cell cultures was investigated by infection of baby hamster kidney cells at a high multiplicity of infection and harvesting the cells at various times post-infection. RNA was extracted from each harvest and used as template material for the PCR. A primer pair of the non-structural protein 1 gene of the South African reference strain of bluetongue virus serotype 4 (SA BTV4 NS1-gene) was used for PCR. The specificity of the PCR for BTV was confirmed by hybridisation using the SA BTV4 NS1-gene as a probe.

The ISH technique was standardised on formalin-fixed paraffin-embedded sections of mouse brain from experimentally infected mice and was then applied on tissue sections of organs of naturally infected sheep. Tissue sections were dewaxed, digested with proteinase K, and hybridised with a radio-active labelled SA BTV4 NS1-gene DNA probe. Hybrids formed were detected by autoradiography.

The PCR and the ISH techniques were both found to be specific and sensitive for the detection of BTV. BTV nucleic acid could be detected using the PCR within 2 h of infecting cell cultures. This rapid technique thus has great diagnostic potential. As the ISH can detect viral nucleic acid in cells of a variety of tissues, it has potential to be used in the further study of the pathogenesis of bluetongue.

# BEHAVIOURAL AND ENDOCRINE CORRELATES OF CONCEPTION IN AN ASOCIAL RODENT

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*Saccostomus campestris*, a cricetid rodent, indigenous of Africa, is generally believed to be a solitarily living species. Earlier studies demonstrated severely aggressive behaviour in the females at all times except when they are sexually receptive, i.e. during pro-oestrus. Early recognition of pregnancy is a controversial topic, usually focussing on the maternal immunological response to conception. The present paper provides a novel approach using behavioural and endocrinological responses to copulation in an asocial species.

Pouched mice were housed under controlled conditions of 14hL : 10hD, 22 $\pm$ 2°C and a relative humidity of ca 55%. Food (rat pellets, Epol, supplemented with apple and sunflower seeds twice weekly) and water were provided *ad libitum*. A male was introduced to each female (n=160) on the morning of pro-oestrus. The following morning, vaginal smears were taken and examined for presence of sperm, and the behaviour of the pairs was noted. As soon as the female showed excessive aggression towards the male, the male was removed.

Blood plasma was sampled from mated as well as nonmated females for measurements of progesterone (n=40), testosterone (n=80) and oestradiol-17ß (n=80). The hormones were measured by means of radioimmunoassay, using established methods specifically evaluated for pouched mice. Ovaries from pregnant and nonpregnant females (n=40) were compared (mass and histology).

The behaviour of oestrous pouched mice towards males appears to depend on whether copulation results in conception. Females that conceived expressed overt aggression towards males, while those who did not, continued to be accommodating during the subsequent phases of that and the following cycle(s). Receptivity continued in these females until conception took place. Males never defended themselves. Plasma progesterone values approximately 12 hours after mating were significantly higher in females that conceived than in those that did not. This difference increased from oestrus to metoestrus. There was no difference in ovarian mass or histology between pregnant and nonpregnant females. Sexual receptivity was not associated with measurable plasma levels of testosterone, and cyclical changes in oestradiol-17ß remained below the detection limit of the assay. The hormonal correlates of these behavioural differences are thus as yet not known. Behavioural changes, however, implied that female pouched mice have the ability to perceive conception as soon as 12 hours after mating.


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