UNIVERSITEIT VAN PRETORIA

FACULTY OF VETERINARY SCIENCE
FAKULTEIT VEEARTSENYKUNDE

14th Faculty Day
de Fakulteitsdag

September 26, 1997

PROGRAMME AND SUMMARIES
PROGRAM EN OPSOMMINGS

Pfizer
Animal Health
Dieregesondheid

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA
FAKULTEIT VEEARTSENYKUNDE, UNIVERSITEIT VAN PRETORIA

14de FAKULTEITSDAG — 14th FACULTY DAY

26 SEPTEMBER 1997

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PFIZER ANIMAL HEALTH / PFIZER DIEREGESONDHEID

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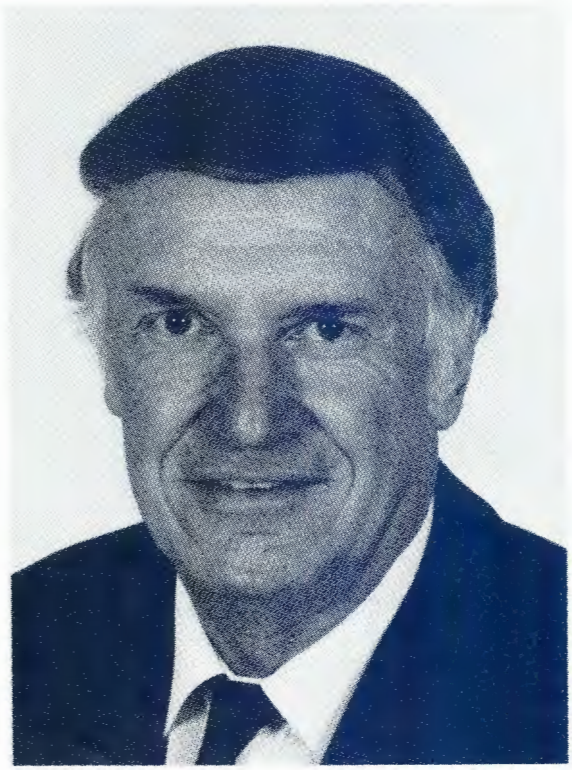
NAVORSINGSPROGRAM (PLAKKATE) / RESEARCH PROGRAMME (POSTERS)

BOODSKAP VAN DIE DEKAAN — MESSAGE FROM THE DEAN

Prof. R.I. Coubrough



The new millennium hurries closer - just over two years remain before this major event occurs and "Millennium Fever" quickens the pulse. The "Year 2000 Problem" is already testing the minds of computer boffins as they toil to prevent the collapse of strategic computer systems when the 1990's turn over to the year 2000. Greenwich, the centre of time and space, is set to be the home of the millennium. Here the east meets the west along the Prime Meridian, and the universal day determines the world's time.

The International Conference of 1884 decreed that the universal day would be a Mean Solar Day, beginning at the Mean Midnight at Greenwich, and that it would be counted on a 24 hour clock. Hence, as the clock ticks closer to midnight on the 31st December 1999, the chimes of "Greenwich Mean Time", radiating out to the east and west, will signal the start of a great moment and herald the dawn of the new millennium. Thousands will throng at the controversial Millennium Dome, built at Greenwich as the focal point of the celebrations of this special event, welcoming the moment with mixed emotion: elation, joy and trepidation.

The events of this moment and the challenges that the millennium will bring to us all cannot be escaped. Even as we take stock of our current research programme this Faculty Day, we must turn our focus to the important research role we will have to play in securing animal and community health into the year 2000. Greater and greater reliance will be placed upon our Faculty to play a pivotal role in solving disease problems which continue to threaten this sub-region in partnership with our neighbours. Many diseases which were prevalent at the turn of the last century are still with us as we move into the year 2000. We must muster the strengths and talents we show today and harness new technologies to eradicate such problems. By defining specific research areas and by focussing our regional research strengths, we will be in a strong position to play a meaningful role in meeting the veterinary challenges of the new millennium as Greenwich Mean Time proclaims the start of the year 2000.

Curriculum Vitae

Prof. David L. Block *PhD, FRAS*

Professor David L. Block, of the Department of Computational and Applied Mathematics at the University of the Witwatersrand, holds a Master of Science degree in relativistic astrophysics and a PhD in astronomy, specializing in the morphology of spiral galaxies. He was elected a Fellow of the Royal Astronomical Society of London at the age of 19, and his first paper (on general relativity) was published by the Royal Astronomical Society at the age of 20.

Professor Block is the author of *Starwatch* (published in English, German and Swedish) and *Our Universe: Accident or Design?*, and is an invited contributor to the *Astronomy Encyclopaedia*. The preface to his second book was written by two Nobel laureates, Arno Penzias (Physics 1978) and Sir John Eccles (Physiology & Medicine 1963). Professor Block is the only South African whose research work has twice been featured on the cover of *Nature*, the world's most prestigious scientific journal.

A recipient of the University of the Witwatersrand's Convocation Distinguished Teacher's Award given to the most distinguished lecturer in the Faculty of Science, Professor Block has been a Visiting Scientist at the European Southern Observatory (Munich and Chile) and the Institute for Astronomy in Hawaii. He was Chairman of the Scientific Organizing Committee for the International Cold Dust-Morphology Astronomy Conference held at the University of the Witwatersrand in 1996 and attended by over 100 international delegates. Professor Block travels extensively, lecturing to audiences of up to ten thousand people per session. He is married to Elizabeth, a lecturer in geography at the Soweto Campus of Vista University, and they have one son, Aaron, aged five.

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

07:45 - 17:30 FOTOGRAFIESE UITSTALLING / PHOTOGRAPHIC EXHIBITION

08:15 - 08:20 WELCOME BY CONVENOR OF THE ORGANISING COMMITTEE / VERWELKOMING DEUR SAMEROEPER VAN DIE REËLINGSKOMITEE - *PROF. F REYERS*

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1. Introduction to veterinary nuclear medicine and the use of the gamma camera
   S.L. Fourie
2. The evaluation of canine leukocyte differential counts on the *Cell-Dyn 3500*
   T. Dippenaar & F. Reyers

08:50 - 08:55 OPENING AND INTRODUCTION OF PROF. D.L. BLOCK / OPENINGSREDE EN VOORSTELLING VAN PROF. D.L. BLOCK - *PROF. R.I. COUBROUGH*, DEAN

08:55 - 10:25 SIR ARNOLD THEILER-GEDENKLESING / SIR ARNOLD THEILER MEMORIAL LECTURE:
"OUR UNIVERSE: ACCIDENT OR DESIGN?" - PROF. DAVID L. BLOCK

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OPVSC REPRESENTATIVE / OPVSK VERTEENWOORDIGER

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   D.H. Volkmann, G.H. Goosen, H.J. Bertschinger, R. Shuttleworth, A. Koekemoer & P.G. Howell

2. Immunocytochemistry of the elephant zona pellucida using anti-pZP antibodies
   H.J. Bertschinger, R.A. Fayrer-Hosken, J.F. Kirkpatrick, J.T. Soley, W. Steffens & M. Ard

3. First *in vitro* produced bovine embryos at Onderstepoort
   T. Arlotto

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5. The pathology of some cases of atypical canine babesiosis
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6. The effect of diminazene aceturate on cholinesterase activity in dogs with canine babesiosis
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PLAKKAATBESIGTIGING EN AANBIEDING / POSTER VIEWING AND PRESENTATION

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7. Zonation of the urea cycle and glutamine synthetase in the ovine liver
   H.C. Rossouw, J.G. van der Walt & M. Nell

8. Ulcerative pododermatitis in free-ranging African elephant (*Loxodonta africana*) in the Kruger National Park
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9. Which is the pregnant horn of an equine afterbirth?
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16:15 - 19:30 AFSLUITING EN SKEMERPARTYTJIE / CONCLUSION AND COCKTAILS

Introduction to veterinary nuclear medicine and the use of the gamma camera

S. L. Fourie

Section of Radiology, Department of Surgery

The Onderstepoort Veterinary Academic Hospital has recently acquired a gamma camera, making available scintigraphic imaging which has a wide range of applications in veterinary medicine and research.

A radioactive atom, usually technetium-99m (Tc), is combined with other chemicals. The radiopharmaceutical which is formed is injected intravenously and delivers the Tc to particular parts of the body. Gamma rays are emitted by the Tc which are detected by the gamma camera. An image is created of the distribution of the radiopharmaceutical, allowing evaluation of both the functional and morphological status of the respective organs/tissues.

Bone imaging is the most commonly performed scintigraphic imaging procedure in veterinary diagnostics. Blood flow and osteoblastic activity can be assessed in detecting early bone disease (e.g. osteomyelitis, acute non-displaced fractures, neoplasia or bone infarcts) before changes are detectable on radiographs. This technique will be widely used in horses with non-specific lameness.

Renal imaging includes functional scintigraphy used to determine glomerular filtration rate, and morphological studies to provide anatomical information.

Porto-systemic shunts can be diagnosed following the intrarectal introduction of Tc, which results in a nuclear porto-angiogram. Other hepatic scintigraphy techniques include morphology and hepatocyte function studies, and the detection of biliary tract obstructions.

Pulmonary perfusion studies can be performed to assess thromboembolic disease in small animals and chronic obstructive pulmonary disease in equines. Ventilation studies are normally used for research.

Myocardial imaging can be used to assess perfusion and ventricular function. Other scintigraphic techniques include splenic, gastro-intestinal and thyroid studies.

With the many possible applications in veterinary medicine and research, the gamma camera is a unique imaging tool as it can assess functional and physiological changes early in disease states and will make a tremendous contribution to the imaging capabilities of our Faculty.

The evaluation of canine leukocyte differential counts on the *Cell-Dyn 3500*

T. Dippenaar & F. Reyers

Section of Clinical Pathology, Department of Medicine

Automated haematology analysers are principally designed for use in human haematology. The *Cell-Dyn 3500* (Abbott Diagnostic Laboratories, Johannesburg) is equipped with a veterinary software package that claims to be capable of performing accurate differential cell counts on common domestic animal species, including dogs.

The purpose of this study was to ascertain whether the differential cell counts are accurate enough for useful clinical interpretation.

The approved standard reference method for leukocyte differential counting and the evaluation of instrumental methods, published by The National Committee for Clinical Laboratory Standards in 1992, was used in this study. Over 100 normal and 100 abnormal blood specimens were evaluated and compared to a 400-cell manual count performed on each specimen.

Although the overall results look promising, there are two problem areas: specific cell types which appear to be "misinterpreted" by the *Cell-Dyn 3500*, specifically basophils and eosinophils as well as monocytes and lymphocytes in some samples; abnormal samples were not always correctly counted, but usually the instrument would flag these samples, warning the technologist that the count was not reliable.

In vitro and *in vivo* testosterone production by an equine granulosa cell tumour

D.H. Volkmann, G.H. Goosen[1], H.J. Bertschinger, R. Shuttleworth, A. Koekemoer & P.G. Howell[1]

Department of Theriogenology; [1]Department of Veterinary Tropical Diseases

Our findings in a case of an early pregnant mare with a granulosa cell tumour (GCT) included very much higher plasma testosterone concentrations (PTC) than have been reported in non-pregnant mares with GCT. During our work-up of this case, we found that PTC only remained at such high concentrations during the first 40-120 days of gestation. This is the period when the endometrial cups secrete the potent gonadotrophic hormone, equine chorionic gonadotrophin (ECG). We thus formulated the hypothesis that ECG stimulates GCT cells to produce more testosterone than they would in the absence of ECG, and that such elevated PTC should be expected in early pregnant mares with a GCT. If we were able to demonstrate the ECG responsiveness of the tumour cells, it could provide an additional diagnostic aid in cases of GCT, even if patients were not pregnant.

A second, non-pregnant mare that presented with a GCT was given 2000 IU ECG intravenously and PTC were monitored for three days. The PTC, however, did not increase in response to the ECG (we assume that this failure to respond was related to the dose of ECG that was much too low). The affected ovary was removed by routine surgical technique and tumour tissue was trypsinised in preparation for cell culture in MEN medium with 10 % foetal calf serum (with $1ml.l^{-1}$ gentamycin). Supernatant medium was removed from the primary cultures after two days and contained measurable quantities of testosterone. Sixty-two subculture tubes were prepared from the primary cell population and treated as follows (31 treatments, two tubes per treatment): two tubes served as controls at the initial time of stimulating the cultures; ten tubes were each stimulated with 50 IU ECG, ten tubes with 5 IU ECG, ten tubes with 0,5 IU ECG, ten tubes with 1 IU HCG, ten tubes with 0,1 IU HCG and ten tubes with 0,01 IU HCG. The supernatant medium from two tubes (one treatment) from each group of ten equally treated tubes was removed at 0,5, 2, 4, 8 and 24 hours post treatment, respectively, and frozen at - 8° C. Stimulation with ECG or HCG failed to induce increased testosterone production.

This case study has provided three novelties: (1) the very high PTC associated with the period of endometrial cup activity; (2) the successful culture of GCT cells *in vitro*; and (3) the detection of testosterone in the tissue culture fluid after two days of culture.

Immunocytochemistry of the elephant zona pellucida using anti-pZP antibodies*

H.J. Bertschinger, R.A. Fayrer-Hosken[1], J.F. Kirkpatrick[2], J.T. Soley[3], W. Steffens[1] & M. Ard[1]

Department of Theriogenology; [1]Department of Large Animal Medicine, University of Georgia, Athens, Georgia, USA; [2]Deaconess Research Institute, Billings, Montana, USA; [3]Department of Anatomy

The aims of the study were to determine the extent of cross reactivity between elephant zona pellucida (elZP) and porcine zona pellucida (pZP), and to provide evidence of the effective potential of pZP vaccine for elephant population control.

The cortical region of ovaries obtained from 12 elephants was cut into 3-5 mm^3 blocks and fixed in Bouin's fluid, processed and embedded in paraffin. The paraffin blocks were sectioned and sections placed on slides and deparaffinized for immunocytochemistry. After blocking in TRIS-buffered saline (TBS) with 3 % BSA, the sections were washed and incubated with rabbit anti-pZP antibody (primary antibody) in TBS with Tween 20 (TBST). Further washing with TBS was followed by incubation with protein A-10 nm gold (secondary antibody) in TBST. After a wash in TBS and then triple distilled water, the immunogold staining was enhanced using a silver enhancement technique. The slides were dried and mounted.

In primary follicles, immunogold deposits were concentrated at the oocyte-granulosa cell junctions. Furthermore, there was diffuse, homogenous staining of the oocyte cytoplasm which exceeded the background and control sections. In secondary and tertiary follicles, there was definitive staining of the zona pellucida. There was no staining of the oocyte cytoplasm. In tertiary and late secondary follicles, there did appear to be some staining of the granulosa cell cytoplasm and the intergranulosa cell spaces.

The clear immunogold staining of the zona pellucida in primary, secondary and tertiary elephant follicles proves that there are shared epitopes between the elephant and porcine zona pellucida. These findings show that antibodies directed against the pZP should recognise elZP and interfere with sperm-zona binding at the time of fertilisation. As in horses and white-tailed deer, the vaccination should result in infertility as long as antibody titres persist. The next phase in a careful, considered approach to the feasibility of elephant contraception, will be to determine the dose of pZP and the optimal adjuvant required to produce detectable anti-elZP antibody levels in elephant. These studies will be followed by field fertility trials.

Research project No. 36.5.251. Approved by the Faculty Ethics and Research Committees and the Research Committee of the National Parks Board of South Africa.

Immunocontraception of African elephant

First *in vitro* produced bovine embryos at Onderstepoort

T. Arlotto

Department of Theriogenology

The *in vitro* fertilization (IVF) of bovine embryos worldwide has enabled cattle farmers to increase the productive capacity of the elite animals in their herds, both dairy and beef. Several techniques aimed at increasing the genetic merit of cattle, when performed in conjunction with IVF, are available: embryo freezing and transport, multiple offspring from valuable donors, twinning, genetic rescue of valuable donors, cloning and embryo sexing. In addition, bovine IVF makes a good model for other, economically less important species. To date, *in vitro* fertilized bovine embryos have not been produced reliably in South Africa. Hence, an IVF laboratory was established to allow South Africans to take advantage of these technological advances.

To initiate embryo production at Onderstepoort, bovine ovaries were collected after slaughter from the Pyramid abattoir. Oocytes were retrieved from the ovaries with the aid of a needle and vacuum pump. After a 24 hour maturation period, the eggs were placed with commercially available frozen thawed semen. After a 20 hour co-incubation of sperm and eggs, the presumptive zygotes were washed of spermatozoa and placed in fresh culture medium. Embryos were cultured for a further seven days, at which time blastocysts were evaluated as an indication of the success of the procedure.

In June 1997, blastocyst production became consistent. During 12 replicates, 245 blastocysts were produced from a total of 244 ovaries (122 cows). A mean of 27 $\pm$5 % (range of 7-64 %) of 1438 oocytes have become blastocysts after IVF.

Variable results are also reported from other international laboratories. Semen from several different bulls was utilized, which affected the *in vitro* fertilization of the oocytes. In addition, the oocytes from different lots of cattle can be of varying quality, all leading to results which may vary from day to day.

In conclusion, the *in vitro* fertilization laboratory at Onderstepoort is able to successfully culture bovine embryos. The next steps will involve the combination of IVF techniques with the aforementioned technologies, particularly the repeated retrieval of oocytes from live donors (trans-vaginal recovery). This will provide South African cattle farmers with additional options for the improvement of their herds.

The acid-base and blood gas disturbances of severe South African canine babesiosis*

A.L. Leisewitz, F. Reyers, J.T. Taylor[1], W.L. Berry & A.J. Guthrie[2]

Department of Medicine;[1]Department of Pharmacology & Toxicology; [2]Equine Research Centre

The acid-base and blood gas disturbances previously described in babesiosis included a metabolic lactacidosis and oxygen content deficit. It has been demonstrated that the outcome of babesiosis is correlated to blood lactate levels. Arterial blood pH is not reflective of the severe base deficit frequently present. Treatment of the acidosis has traditionally been intravenous alkali ($NaHCO_3$) administration. Investigations have shown that acid-base and blood gas changes can be reversed with whole blood transfusion. It has become evident that severe babesial disease is never associated with simple acid-base disturbances. In a detailed study of 15 cases, the most consistent imbalances involved were metabolic lactacidosis, hyperchloraemic acidosis and respiratory alkalosis.

Similar changes have been reported in canine endotoxaemia, in an experimental rat model of inflammation, and in human sepsis. Just as human malaria has been likened to endotoxaemia, canine babesiosis has striking similarities to both of these conditions. It is a clear example of the systemic inflammatory response syndrome which occasionally progresses to multiple organ dysfunction. Mortality is correlated with renal failure, lung failure and base deficit.

The oxygen dissociation curve in canine babesiosis is shifted to the right. This shifting is, however, compromised by the endogenous production of carbon monoxide, probably by haem-oxygenase salvaging of haemoglobin.

**Research project No 36.5.266. Approved by the Faculty Ethics and Research Committees.*

The pathology of atypical canine babesiosis

A. Pardini & N.P.J. Kriek

Department of Pathology

The haemoprotozoan parasite, *Babesia canis*, typically causes a haemolytic syndrome in susceptible dogs. In Africa, clinical babesiosis is common and approximately 30 % of clinical cases present with severe complications (so-called atypical forms).

At necropsy, atypical babesiosis is characterised either by systemic lesions resembling septic shock (haemoconcentration form) or by organ predilection, thereby resulting in cerebral, cardiac, pneumonic, renal, gastro-intestinal and other forms, with associated organ failure. The macroscopic features include multifocal cerebral petechiation to ecchymoses (cerebral babesiosis), multifocal subendocardial ecchymoses, acute interstitial pneumonia, moderate to severe nephrosis with haemoglobin pigmentation, or severe hyperaemia of the small intestine with enterorrhagia.

Microscopic features of brain lesions include capillaries and venules packed with parasitised erythrocytes, adherence of parasitised erythrocytes to endothelium, perivascular oedema and haemorrhage, and, in severe cases, thrombosis, ecchymoses, vasculitis, malacia and neutrophil infiltration. These lesions show some similarity to human cerebral malaria, and similar pathogenic mechanisms are likely to be present.

The effect of diminazene aceturate on cholinesterase activity in dogs with canine babesiosis*

R.J. Milner, F. Reyers, J.T. Taylor[1] & J.S. van den Berg

Department of Medicine; [1]Department of Pharmacology & Toxicology

It has been suggested that the side effects associated with the use of diminazene aceturate in canine babesiosis are due to the inhibition of cholinesterase (ChE). A clinical trial was designed to evaluate the effects of diminazene aceturate and its stabilizer antipyrine (Berenil®) on plasma pseudocholinesterase (PChE) and red blood cell acetylcholinesterase (RBC AChE).

The trial was conducted on naturally occurring (uncomplicated) cases of babesiosis ($n=20$) which were randomly allocated to groups receiving a standard therapeutic dose of diminazene aceturate with antipyrine stabilizer (treatment, $n=10$) or antipyrine alone (control, $n=10$). Blood was drawn immediately prior to and every 15 min for one hour after treatment. The blood sample was centrifuged to separate red blood cells from plasma and stored (one month) before being batch processed to determine plasma PChE and RBC AChE. Plasma PChE showed a decrease between 0 min and 60 min within the treatment group ($p<0,05$). The decrease was, however, only 4 % and this was judged to be of no clinical significance. No statistically significant differences were found between the treatment and control groups at any of the time intervals for PChE. There was an increase in RBC AChE activity at 15 min in the treatment group ($p<0,05$). Again, this was judged to be of no clinical significance. No significant differences for RBC AChE at any time interval were found between the treatment and control groups.

In view of the difference in PChE, samples from additional, new cases ($n=10$) were collected to identify the affect of the drug over 12 hours. No significant depression was identified over this time interval. The results suggest that the underlying mechanism in producing side-effects, when they do occur, is unlikely to be through ChE depression.

Research project No 36.5.235. Approved by the Faculty Ethics and Research Committees.

Zonation of the urea cycle and glutamine synthetase in the ovine liver*

H.C. Rossouw, J.G. van der Walt & M. Nell

Department of Veterinary Physiology

The liver perfusion technique has yielded data that suggests that the liver plays a major role in acid-base balance homeostasis; e.g. perivenous glutamine synthetase acts as a high affinity scavenger for the ammonia which escapes periportal detoxification by urea synthesis; glutaminase acts as a pH- and hormone-controlled mitochondrial ammonia amplification system; and that glutamine cycling is under complex metabolic and hormonal control involving flux changes through both periportal glutaminase and perivenous glutamine synthetase. All of these factors support the controversial contention that the liver acts as a major pH homeostatic organ. There is, however, to the best of our knowledge, no documented evidence of the zonation of N-metabolising pathways in sheep.

Young adult sheep ($n=18$) were kept on conventional grazing, supplemented with lucerne/tef hay feeding. Under general anaesthesia, a paracostal incision was made in the left flank about 3 cm caudal to the last rib, starting at the lumbar margin and extending to beyond the ventral midline. Suitable vascular snares were loosely placed around the vessels leading to and from the liver. A cannula was inserted via the portal vein into the branch leading to the small, caudate lobe, and secured. The cannula was used to perfuse the lobe with heparinised, well-oxygenated physiological buffer at 39° C. The sheep was then euthanased, the snares tied off and the liver immediately removed. A second cannula was secured in the vessel draining the caudate lobe.

The lobe was suspended in Krebs-Henseleit buffer (39° C) throughout the experiment and gassed with carbogen (95 % O_2 plus 5 % CO_2). The buffer was pumped through the lobe at rates varying between 60 and 100 $ml.min^{-1}$ in both the antegrade and retrograde directions. Samples (6 ml) were collected, and sub-samples were also separately drawn for blood-gas analysis (ABL3) at 20 min intervals. Hydrostatic pressures were monitored on the upstream catheter. To determine the degree of anaerobic damage, samples of the liver were taken for histopathological examination at the beginning and at the end of the perfusion.

The zonation of N-metabolising pathways in sheep was tested by changing the direction of perfusion and by changing the content of the perfusate. Ammonium chloride and/or glutamine were added to the buffer, with and without the presence of sulfoximine (glutamine synthetase inhibitor). Metabolites assayed included ammonia, urea, glutamine, glutamate, lactate and pyruvate.

The liver abstracted about twice the amount of ammonia from the perfusate as it produced urea. When ammonia was excluded from the perfusate, the amount of urea produced declined by an order of magnitude, reflecting the endogenous rate of amino acid catabolism. The rate of production was not as sensitive to the direction of flow as that found in rats, casting some doubt on the zonation of hepatocytes in sheep. However, glutamine output and glutamate uptake were very sensitive to flow direction, corroborating the rat data. The inclusion of sulfoximine eliminated this response completely, thereby confirming that it was due to the presence of glutamine synthetase in the perivenous hepatocytes.

**Research project No 36.5.284. Approved by the Faculty Ethics and Research Committees.*

Ulcerative pododermatitis in free-ranging African elephant (*Loxodonta africana*) in the Kruger National Park

D.F. Keet, D.G. Grobler[1], J.P. Raath[1], J. Gouws[2], J. Carstens[2] & J.W. Nesbit[3]

Office of the State Veterinarian, Kruger National Park; [1]National Parks Board, Skukuza; [2]Department of Veterinary Tropical Diseases; [3]Department of Pathology

A clinical, pathologic and bacteriologic investigation into the occurrence of severe lameness in 13 free-ranging adult African elephant bulls in the Letaba Land System in the Kruger National Park was conducted during the period April 1993-October 1995. A single, large ulcer in the sole of at least one front foot, occasionally both front feet and, in three cases, all four feet were present. Microscopically the lesion was categorised as severe chronic active ulcerative bacterial pododermatitis complicated by hypersensitivity/septic vasculitis. A variety of bacteria were isolated from these lesions as well as from the regional lymph nodes in four cases. *Streptococcus agalactiae* was the most consistent isolate while *Dichelobacter nodosus*, the only microorganism known to be involved in the pathogenesis of diseases of the feet in ruminants, was isolated in two cases.

Contributory factors include the occurrence of the outbreak in the Letaba Land System that is dominated by shrub mopane (*Colophospermum mopane*); the occurrence of the outbreak during a drought; the restriction of the lesion to adult elephant bulls; and, the predilection of the lesion in the front feet may have predisposed to the development of the outbreak.

Which is the pregnant horn of an equine afterbirth?

D.H. Volkmann

Department of Theriogenology

During the routine examination of the mare's afterbirth, attention is always given to the correct identification of the "pregnant" horn of the allantochorion (AC). The latter is assumed to be that part of the AC in which the foetus develops during pregnancy. Identification of the pregnant horn involves the laying-out of the AC in an F shape, which always brings the pregnant horn into the position of the upper arm of the F. Invariably, this arm of the F is larger, has a smoother surface and a much more oedematous tip than the lower one. Usually, but not always, the attachment of the umbilical cord is at the base of the upper arm of the F. The "unusual" attachment of the umbilical cord at the base of the lower arm of the F has been described as an incidental finding without further diagnostic significance. Remnants of the endometrial cups are usually (not always) present on the allantoic surface of the AC. If present, they are always found near the attachment of the umbilical cord. The endometrial cups develop between Days 34 and 45 of gestation, at a time when the conceptus is still entirely located inside a single uterine horn (the "pregnant" horn). The spatial association between the endometrial cup remnants and the umbilical cord attachment justifies the conclusion that the umbilical cord attaches on that portion of the AC that lies in the uterine horn in which the conceptus originally fixed (Day 16 of gestation).

During the last ten years, the author has examined several hundred equine afterbirths. Infrequently, but regularly, the umbilical cord attached on the smaller, lower arm of the F of the AC. In six such cases, the remnants of the endometrial cups were clearly identifiable, and in all six cases they were positioned near the site of the apparently abnormally positioned umbilical cord attachment.

The exact frequency of this unusual arrangement of the structures of the equine AC cannot be drawn from this report, but it is obvious that: (1) the spatial association between endometrial cup remnants and umbilical cord attachment is always the same; (2) the umbilical cord may sometimes attach at the base of what has hitherto been described as the non-pregnant horn of the AC; and (3) in cases where the umbilical cord attaches to the smaller horn of the AC, that horn must be designated the pregnant one, because it is the one in which fixation of the embryo first occurred.

In conclusion, the physical appearance or size of the horns of the AC should not be used to identify the pregnant horn. Much rather, the horn that contains the umbilical attachment should be used to correctly identify the pregnant horn of the AC. Apart from correcting a false tradition, this report lends support to the observation of practitioners that equine pregnancies sometimes "migrate" from one uterine horn to the other. The findings reported here suggest that the foetus can first develop inside one uterine horn (the pregnant one) and later during gestation continue its growth in the other horn, resulting in greater expansion of the non-pregnant horn.

Helminth parasites of dogs in a resource-limited community in South Africa*

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The aim of the study was to determine the socio-economic role of dogs in resource-limited communities, to define their health status and extent of veterinary care, to investigate the occurrence of parasites within these communities (with special reference to helminth parasites), and to assess the zoonotic helminth potential in order to develop appropriate means of internal parasite control. Seroprevalence studies involving *Toxocara* spp. alone in humans that were published in 1988, were found to be as high as 80 % in children and 47,5 % in adults in Venezuela. South African figures are outdated, but the latest (1979) indicated prevalence of 31 % for *Toxocara canis*, 11,9 % for *Ancylostoma caninum* and 1,5 % for *Echinococcus* spp. in the Pretoria area. This is disturbing, as 80 % of positively diagnosed cases occurred in urban areas.

Many helminth parasite species found in dogs can seriously affect humans as their definite hosts, intermediate hosts, potential hosts or opportunistic hosts. Detrimental effects of these parasites are in many cases unapparent, but more pronounced in children; they include stunted growth, anaemia, asthma, seizures and ocular damage.

Information on the socio-economic role of animals in these communities was gathered by means of a semi-structured interview and a questionnaire. Samples collected included the following: faecal samples (fresh) collected per rectum for demonstration of nematode eggs, blood films (smears) in order to detect microfilariae and protozoa, heparinised and/or EDTA blood samples for acid phosphatase staining of microfilariae, and adhesive tape swabs of perianal skin for demonstration of tapeworm eggs. Dogs were also examined clinically in order to determine health status and body condition, and to collect external parasites.

Of 58 faecal samples collected, 54 (93 %) contained eggs of *Ancylostoma* spp., whereas eggs of *T. canis*, *Toxascaris leonina*, *Dipylidium caninum* and a taeniid spp. were found only in one sample each (the eggs of *Taenia* spp. and *Echinococcus* spp. can not be distinguished microscopically from one another). Cestode eggs, filarial nematodes and blood protozoa were not found during the examination of adhesive tape swabs, membrane filtered blood or blood films respectively.

The high incidence of *Ancylostoma* spp. in the dogs in this community, and the presence of other zoonotic helminths is significant both from animal and public health perspectives. The presence of these dog helminth parasites in this community stimulates further studies. Such studies might address the impact of zoonotic helminths in resource-limited communities, or the correlation with occurrence of conditions such as cutaneous larva migrans in humans.

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Ultrastructural differences in mammalian platelets

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Comparative haematological studies between different mammalian species are valuable since it can be deduced that features common to all are probably biologically important. Platelets are found in the blood of all mammals and serve the same haemostatic function. There are, however, species differences in response to aggregation, possibly because of different surface glycoproteins. Species differences in platelet size have been observed but not quantitated to date. In this study, the ultrastructural differences in platelets of three mammalian species were studied and documented.

Blood was drawn from 20 buffalo and three rhinoceroses in the Kruger National Park and compared with human blood platelets. Acid citrate dextrose was used as anti-coagulant. The platelet-rich plasma was fixed in 2,5 % glutaraldehyde and post-fixed in 0,5 % osmium tetroxide. Following dehydration in an acetone series, the samples were embedded in Quetol, sectioned, stained and viewed with a Philips 301 transmission electron microscope.

The internal platelet structure of the different species had similar morphology, but there were differences between the species. The human platelets showed signs of activation, with the production of pseudopodia and the centralization of granules, whereas the animal platelets showed little or no activation. Human platelets appear bigger, but with smaller granules than the animal platelets. The granules of the rhinoceros platelets demonstrated a large variation in morphology and were difficult to categorise, while the buffalo had prominent and large α-granules. A marked difference between the animal and human platelets was the presence of many dense bodies in the animal platelets. Other features of the buffalo platelets were the absence of the open canalicular system, but the presence of a prominent ring of microtubules. The rhinoceros platelets were characterized by an abundance of glycogen. The three species studied presented the same basic platelet morphology (anuclear cytoplasm with granules scattered throughout together with some form of tubular system). There were, however, distinct differences between the species. The variation in morphology was such that in mixed preparations of platelets, the platelets of each species could be easily identified.

Ultrastructural morphometric observations of different mammalian platelets

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The blood platelets in circulation are heterogeneous with respect to platelet count and size. Normal values for a specific species can vary between individuals of the species. Some people believe the platelet size to vary with age, while others believe platelet formation to be responsible for size variation. Qualitative fine-structure examinations have been carried out on human platelets by a number of investigators as such information is of value in assessing normal and pathological conditions. Information regarding the ultrastructural morphometric observations of the sectioned platelets of wild animals is not readily available.

Comparative studies were done on the platelets of six humans, six cattle, 20 buffalo, six white rhinoceros and ten cheetah. Image analyses were performed on micrographs taken of the platelet sections prepared for transmission electron microscopy. Measurements were made directly on the micrographs. Parameters measured included area, external perimeter, minimum and maximum projections, width and aspect ratio. At least 100 platelets per species were measured and only platelets where the microtubules could be seen as distinct circles at both ends of the platelets, were included.

The first five parameters revealed a similar pattern regarding the direct measurements of the platelets. The rhinoceros platelets were the smallest, followed by the bovine and human platelets. Cheetah platelets were the biggest. The only exception was the aspect ratio, which is an indication of the platelet shape. The cattle had the lowest aspect ratio, indicating that these platelets were the most spherical.

The determination of accurate values for these measurements is important for a clear understanding of platelet morphology. These values provide additional baseline information for interspecies comparisons and values against which sick animals of the same species can be assessed as certain diseases are known to alter platelet morphology.

Formaldehyde gas sterilisation of surgical instruments*

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Although it is not widely used, formaldehyde gas is used successfully by some practitioners for the cold sterilisation of surgical instruments. The advent of aqueous glutaraldehyde disinfection in the 1930's caused a waning of earlier interest in formaldehyde. However, apart from sterilisation of surgical instruments, there is still a specific role for gas sterilisation. Certain equipment (electrical motors and cables, some ophthalmic and dental instruments) are incompatible with steam or chemical sterilisation.

Formaldehyde is not a human carcinogen but a normal cellular metabolite, and levels in mammalian cells normally range from 1,5 to 15 ppm. Human exposure of up to 15 ppm of formaldehyde in air has therefore little effect on intracellular levels and suggests that the rate of endogenous production and metabolism must be greater.

The study was undertaken to evaluate the efficacy of formaldehyde gas in sterilising surgical instruments in closed containers under differing temperatures and humidity. Following the introduction of paraformaldehyde powder to the desiccators, the formalin gas concentration was 4 ppm after one hour and 14 ppm after 24 hours. The results of the microorganism cultures indicate that formalin gas at 14 ppm with a relative humidity exceeding 42 % and a temperature above 10° C, will adequately sterilize instruments in 24 hours time. This corresponds to average summer and winter weather conditions in many parts of the world. The temperature and relative humidity prevailing in a group of jars constitute the optimal conditions for formaldehyde's antimicrobial effect. Formaldehyde remains effective under less than ideal conditions of temperature and relative humidity.

Any suitably sized plastic, glass or metal container with a tight-fitting lid can be used as a gas sterilisation chamber for surgical instruments, electrical motors and cables, rubber tubes and certain suture materials.

Formaldehyde gas sterilisation is an economical technique due to the relatively cheap equipment and running costs. Virtually any container with a well-sealing lid that can accommodate the specific instrumentation can be used. A full range of plastic containers with snap-on or screw type lids is readily available from local supermarkets or hardware stores.

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Genetic distance in Nooitgedacht and Basuto Pony

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The Nooitgedachter was developed after the Basuto pony, which originated out of the Cape horse in Lesotho by the 1940's. Because of previous inbreeding, very selective breeding was required. Upgrading was done separately in the two groups with different breeds. The purpose of the study is to construct dendograms of genetic similarity.

Blood samples were collected from 30 pure bred Nooitgedachters in Mpumalanga Province and 30 Basuto ponies in Lesotho. Acetate dextrose tubes (7 ml) were used for blood collection. The blood was analysed according to conventional blood typing. Factors tested for are according to the minimum requirements set by the International Society for Animal Genetics. The data was analysed according to Biosys 1 (University of Illinois).

Heterozygosity values in both breeds were still very high. The genetic distances according to Nei are very small. The dendogram may reflect the historical and phylogenetic record, however, allelic frequencies and Nei's "D" can be affected by mutation, natural selection, artificial selection, founder effect or geographic isolation, which could influence the interpretation.

The inbreeding in the two different breeds did not influence the genetic variation. The different breeds that were used for upgrading did not influence the similarity between the breeds. Other South African breeds will be tested against these breeds.

Antigenic type distribution of canine and feline parvoviruses in dogs and cats in southern Africa

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Canine parvovirus (CPV) and feline panleukopenia virus (FPV) are closely related viruses that are classified as the feline parvovirus subgroup within the family Parvoviridae. They are important pathogens for their respective hosts, and they also represent an important virus system to study viral evolution. CPV emerged in the late 1970's, most likely as a variant of the well known FPV, and continued to evolve and adapt to the new canine host. During that adaptation it gave rise to new so-called antigenic types, namely CPV-2a and CPV-2b. These new types have completely replaced the old type in Europe, Japan and the USA. Both CPV-2a and CPV-2b are able to replicate in cats, whereas the original type CPV-2 only replicates in dogs. The reason for their evolutionary advantage is not known, but acquiring the feline host may have been an important factor.

Viruses from faecal specimens were isolated in cell cultures and confirmed by haemagglutination. Viruses were typed by using monoclonal antibodies.

We here describe for the first time the new antigenic types in samples from dogs in southern Africa. The antigenic typing of a total of 14 isolates from dogs from Namibia and South Africa revealed twelve CPV-2b and two CPV-2a isolates. The original type CPV-2 was not found. The complete replacement of the original type conforms to the situation in other countries of the world, but the high percentage of CPV-2b isolates (85 %) differs from most other countries where CPV-2a is the predominant antigenic type. The only cat isolate examined was a true FPV-type virus. Further analysis of African parvoviruses, including extending the serological typing to other domestic and free-ranging carnivores may reveal additional meaningful information.

Prevalence and biodiversity of strongyle parasites in donkeys in South Africa*

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Eleven million people live in the rural areas of South Africa and lack dependable and affordable transport. The use of donkeys for traction or draught fulfil many daily needs for these communities e.g. transportation and agricultural practices. Little is known about the nematode parasites of South African domestic equids, particularly donkeys. Donkeys are host to large numbers of helminths, many of which can adversely affect their health. This study aims to inventory the biodiversity of worm species harboured in the ventral colons of two donkeys in South Africa.

Two adult donkeys from Mpumalanga Province were obtained for an integrated study at the University of Pretoria, Faculty of Veterinary Science, Onderstepoort. Donkey one (female) was killed in July 1996 and donkey two (male) in October 1996. The gastrointestinal tracts and the organs were examined according to previously described methods. Aliquots from the ventral colon included two 1/100 which were examined microscopically, and one 8/100 examined macroscopically. Worms were recovered and identified to species level and total estimates were made.

Three large and nine small strongyle (cyathostome) species were identified. Seventy-eight percent of the total number of adult worms were recovered from the ventral colon ingesta and 22% from the ventral colon wall wash. The most abundant cyathostome species in both donkeys were *Cyathostomum montgomeryi*, *Cylicocyclus auriculatus* and *Cylicocyclus sp.a*. The large strongyles were present in fewer numbers. Furthermore, approximately 25 % of the total worm count in the two donkeys (30 406) were fourth-stage larvae, and moulting fourth-stage and fifth-stage larvae. This is the first complete quantitative study on the ventral colon of donkeys in South Africa. Further studies will include larger numbers of donkeys and the entire gastrointestinal tract.

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