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FACULTY OF VETERINARY SCIENCE

16th Faculty Day

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PROGRAMME AND SUMMARIES

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

16th FACULTY DAY

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CONTENTS

Research Programme – Posters

CURRICULUM VITAE

DR. DW VERWOERD

Dr. Verwoerd qualified as a veterinarian in 1955 at the Onderstepoort Veterinary Faculty, obtained a DSc, in Biochemistry in 1963 and a DVSc in 1969, both from the University of Pretoria. Since 1956 he was attached to the Onderstepoort Veterinary Institute as researcher with intervals of 3 years as Alexander von Humboldt research fellow in Germany, from 1960 to 1963 and as Elaenor Roosevelt fellow in the US from 1972 to 1973. In 1964 he founded a Molecular Biology division at the OVI, the first of its kind in South Africa. He pioneered molecular studies on viruses, first concentrating on the economically important bluetongue virus and later on the virus causing jaagsiekte, as a model for cancer-causing viruses. In 1985 he was awarded the Gold Medal of the South African Veterinary Association and in 1987 the Havenga Prize of the S.A. Akademie vir Wetenskap en Kuns for his research achievements. In 1988 he became the tenth Director of the OVI and served in this capacity until his retirement in 1998. He was President of the South African Microbiological Society from 1981 to 1982, served on the Advisory Board of the Virology Division of the International Union of Microbiological Societies, on the editorial boards of various scientific journals and on the National Committees for Microbiology, Biochemistry and Biotechnology. From 1993 to 1998 he was chairman of the OIE Regional Collaborating Centre for Africa and received the Meritorious Award of the OIE in 1998.

CURRICULUM VITAE

DR. JOHN ELLIS, DVM

Dr. John Ellis is a Professor in the Department of Veterinary Microbiology, Western College of Veterinary Medicine at the University of Saskatchewan where he supervises the diagnostic virology laboratory, teaches veterinary students and conducts research on vaccine efficacy in a variety of species. Prior to working at the University, Dr. Ellis worked in a mixed animal practice on the Navajo Reservation, where he coordinated the SCAVMA Native American Project Externship Program. Dr. Ellis also completed pathology residency including research comprised of chronic lung disease of sheep in Colorado, Wyoming and in the Peruvian Andes. Dr. Ellis then went on to spend two-and-a-half years in Nairobi, Kenya, East Africa at the International Laboratory of Research on Animal Diseases where he was a postdoctoral fellow studying basic bovine immunology and immune responses to haematoprotozoan parasites in cattle. Dr. Ellis also was a diagnostic pathologist at the University of Wyoming where he conducted research on BVD and BRSV.

Dr. Ellis graduated with a Doctorate of Veterinary Medicine from the University of Illinois in 1979. He received a PhD in Comparative Pathology at Colorado State University.

PROGRAMME

7:30 - 8:00 REGISTRATION AND COFFEE/TEA

MASTER OF CEREMONIES - *PROF. F. REYERS*

8:00 - 8:10 WELCOME AND OPENING ADDRESS - *PROF. N.P.J. KRIEK*, DEAN

RESEARCH PROGRAMME: SHORT COMMUNICATIONS
SESSION CHAIRMAN: *PROF. A.J. GUTHRIE*

8:10 - 9:10

1. The first case of equine nocardioform placentitis in South Africa
   Volkmann, Williams J.H. and Henton

2. Is the inertia of an entrenched biomedical vocabulary an impediment to progress?
   Williams M.C.

3. The effect of road transportation on external water and electrolyte balance of conditioned horses
   Van den Berg, Guthrie, Meintjes, Nurton, Adamson, Travers and Lund

4. Immune responses in horses experimentally infected with African horse sickness virus
   Chitula, Venter E.H. , Howell and Guthrie

9:10 - 9:30 AN UPDATE ON BOVINE VIRAL DIARRHOEA - *PROF. J. ELLIS*

9:30 - 10:15

5. Effects of anionic salts in a prepartum dairy ration on serum and urine calcium
   Van Dijk and Lourens

6. Radiography of the thoraco-abdominal cavity of the ostrich (*Struthio camelus)*
   Wagner and Kirberger

7. Hysteroscopy in the bitch
   Gerber and Nöthling

10:15 - 10:25 "LECTURER OF THE YEAR" AWARD - *OPVSC representative*

10:25 - 10:45 TEA AND VIEWING OF POSTERS

10:45 - 11:30 SIR ARNOLD THEILER MEMORIAL LECTURE:
"THE MOLECULAR REVOLUTION IN BIOLOGY AND VETERINARY SCIENCE"
DR. D.W. VERWOERD

RESEARCH PROGRAMME: SHORT COMMUNICATIONS
SESSION CHAIRMAN: *PROF. G.F. BATH*

11:30 - 13:15

8. Liver perfusion with hyperosmotic media stimulates glutamine and urea synthesis
Ali, Rossouw and van der Walt

9. Vector competence of selected South African *Culicoides* species for the Bryanston serotype of equine encephalosis virus
Venter G.J. , Groenewald, Paweska, Venter E.H. and Howell

10. The use of community action plant to facilitate grassroots development of animal health and production programmes
Stewart and Wade

11. Anaesthetic sparing effect of midazolam in propofol induced and isoflurane maintained anaesthesia during ovariohysterectomy in dogs
Stegmann and Bester

12. Evaluation of the diagnostic usefulness of serum uric acid concentration in the assessment of liver function
Reyers, Myburgh and Goddard

13. Pharmacological enhancement of emission and ejaculation in cattle: a look at imipramine hydrochloride
Cordel, Keegan and Bertschinger

14. Fertility of bitches after intravaginal insemination with frozen-thawed sperm extended with either prostatic fluid of protein-free TALP prior to insemination
Shuttleworth and Nöthling

13:15 - 14:30 LUNCH

14:30 VIEWING OF POSTERS

The first case of equine nocardioform placentitis in South Africa

DH Volkmann[1], JH Williams[2], MM Henton[3]

[1]College of Veterinary Medicine, Cornell University, Ithaca, NY 14853
[2]Department of Pathology, Faculty of Veterinary Science, Onderstepoort, 0110, RSA
[3]Dept of Microbiology, ARC- Onderstepoort Veterinary Institute, 0110, RSA

Nocardia spp have sporadically and rarely been recorded worldwide as causes of equine infertility (1), endometritis (2) (3), abortion (4) and amongst the genital flora of clinically healthy mares (5). Placentitis caused by a nocardioform actinomycete which is non acid-fast and related to *Nocardia* sp, has emerged as a "new" disease in the late 1980's causing loss of hundreds of foetuses in Kentucky, USA, and recently also beyond its borders (6) (7) (8) (9). The infection has been associated with abortions, stillbirths and early neonatal deaths, while some foals remain uninfected despite placental infection. Nothing is yet known about the pathogenesis, epidemiology or mode of transmission of the disease.

In February 2000, a near fullterm aborted Friesian foal and its membranes, from the Pretoria area, were presented for examination to the Department of Pathology of the Onderstepoort Veterinary Faculty of the University of Pretoria (10). The foal was normal, it had died intra-partally due to hypoxia from premature placental separation, and various tissue specimens from the viscerae cultured negative at the Onderstepoort Veterinary Institute for any significant microbial growth.

The chorion showed a focal, well-delineated, circular uterine body area of purulent placentitis from which Diff-Quik stained impression smears clearly showed numerous beaded, filamentous, branching organisms intra- and extra-cellularly in the predominantly neutrophilic exudate. This organism was subsequently cultured on chocolate agar from samples of the lesion. It was found to be gram positive and non acid-fast, and superficially conforms to the description of *Saccharomyces cryophilis*. A related organism found by Edwards and Simpson (1988) (11), from an aborted equine fetal lung and membranes was classified by the Actinomycete Laboratory at CDC to be *Rhodococcus rubropertinctus*. Final identification of the South African isolate and comparison with the Kentucky isolate is to be performed by Dr Neil Williams of the Livestock Disease Diagnostic Center at the University of Kentucky.

Histopathological examination revealed an abrupt, locally-extensive, acute to subacute, proliferative, plasmalymphocytic chorionitis with copious intra-lumenal purulent exudate; with the presence of many of the described organisms in the exudate and intra-cellularly in the villous epithelial cells. The organism stained strongly gram positive, periodic acid schiff positive and Fite-Faraca (a modified acid fast stain specifically for *Nocardia* sp) negative.

A saline uterine flush was performed on the mare four days after foal heat, and a few of the nocardioforms found in the placentitis were cultured from this specimen. After a two week course of oral trimethoprim-sulpha, based on antibiogram sensitivity performed on the original culture, a post-treatment flush cultured negative. A semen sample collected from the Dutch-imported Friesian sire of the foal, standing at stud in the Lichtenburg area of North West Province, cultured negative for the nocardioform. He has sired more than a hundred foals in 4 years since arriving in RSA.

Is the inertia of an entrenched biomedical vocabulary an impediment to progress?

MC Williams

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Notwithstanding the tremendous contributions that have been made with regard to the terminology of lesions by such intellectual giants as Rudolf Virchow, many of these terms are past their "sell-by date". Much of our pathomedical vocabulary stems from the histological study of lesions that dates from the mid-nineteenth century. Newer concepts of lesion pathogenesis, and a much more fundamental understanding of the biology (particularly molecular biology) of organisms and their reactions to injury, have rendered some terms incorrect or, at least, inaccurate. For some lesions there are not even single terms that can be used to denote them. The above statements will be illustrated by means of examples, and the possible effects that these misconceptions may have on scientific progress will be briefly appraised.

The effect of road transportation on external water and electrolyte balance of conditioned horses

J. S. van den Berg[1], A. J. Guthrie[2], R. A. Meintjes[3], J. Nurton[2], D. Adamson[2], C. Travers[2] & R. Lund[2]

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[2]Equine Research Centre, Faculty of Veterinary Science, University of Pretoria
[3]Department of Physiology, Faculty of Veterinary Science, University of Pretoria

Transportation, strange surroundings and a change in management may all combine to reduce water intake in horses transported by vehicle. Apart from decreased water intake, horses may lose fluids via sweating, urine, evaporative loss from the respiratory system and via the faeces. The objectives of this trial were to measure the water and electrolyte intake and loss of horses during road transportation in relatively hot environmental conditions.

Six adult, Thoroughbred horses that were in full training were used in a balanced crossover design. This model was selected to simulate the type of horse that is transported most often over long distances in South Africa. The horses were randomly assigned to one of two treatment groups. One group were transported, while the second group served as controls. A period of one month was allowed before the crossover. Horses in the transport group were transported by tarred road in a circular route over 600 km. This allowed the standardization of measurements and the use of the same instruments (scale & laboratory) in both groups.

Data were collected in each treatment group, before, during transportation that lasted for eight hours (transport phase) and for six hours after the transportation (recovery phase). The following data were collected or calculated: Water and electrolyte (sodium, potassium and chloride) intake and output, changes in body weight, haematocrit, serum concentrations of protein, albumin, creatinine, sodium, potassium, chloride, osmolality as well as feed consumption.

Although water was always available, the transport group failed to drink during transit. Based on bodyweight, the transported horses were 3% dehydrated at the end of transit. This loss in bodyweight was corrected within one hour after their return due to a higher water intake compared to control horses. The feed intake in the transported horses was unaffected during travelling, but was decreased for six hours following transportation. The output of water via the urine remained the same in the two treatment groups during and following transportation. The faecal output of water decreased in the transported horses and remained lower than the control group for six hours following transit. Electrolyte intake was unaffected by transportation. Sodium and potassium loss via the faeces and urine were similar in the two treatment groups, whereas potassium output was decreased in the transport group during the recovery period of the study. Apart from the decrease in bodyweight the dehydration in the transport group could not be detected clinically. The haematocrit and serum potassium values were unchanged, while serum protein, albumin, creatinine, sodium, chloride and osmolality measurements were elevated at the end of transportation.

It was concluded that transportation by road affected the water and electrolyte balance of conditioned horses for a period up to six hours after travelling.

Immune responses in horses experimentally infected with African horse sickness virus

JCA Chitula[1], EH Venter[1], PG Howell[1], AJ Guthrie[2]

[1]Department of Veterinary Tropical Diseases
[2]Equine Research Centre

African horse sickness (AHS) is an arthopod-borne viral disease of solipeds, causing high mortality in susceptible populations of horses and occasionally other equids. The disease is endemic in South Africa and its importance has increased with international trade in horses and horse racing.

In this study two groups of two AHS-naïve horses were identified. In group one (horse 1 and horse 2), the horses received 10^4 PFU/mL of AHS cell culture-adapted virus by intravenous inoculation, and then challenged 35 days later with 10^6 PFU/mL of the same virus. In the second group (horse 3 and horse 4), the horses received virus-free cell culture supernatants in the first part of the experiment, and then 10^6 PFU/mL of the virus suspension 35 days later. Only horse 4, in the second group, exhibited clinical signs of AHS virus infection, characterized by high temperature (average of 40°C), 10 days post-inoculation for a period of 6 days.

The concentrations of virus in the blood were determined by PFU assay and by a capture-ELISA for confirmation of AHS virus infection after passages in BHK cells. High viraemia levels ($>12x10^3$ PFU/mL) were detected 11 days post-inoculation reaching a maximum of $23x10^3$ PFU/mL on day 14 followed by a sharp decline on day 15. Using an AHS virus-specific polyclonal serum, the capture-ELISA gave high OD readings (OD>1.1) in samples collected between 13 to 19 days post-inoculation, confirming the presence of AHS virus antigens during this period. Using a competitive ELISA, seroconversions of 60-100% inhibition were detected 12 to 18 days after the second challenge inoculation in horses 1, 2 and 3, and from 18 to 30 days in horse 4.

In order to identify specific patterns of the immune responses to African horse sickness, monoclonal antibodies were used in an isotype-specifc ELISA to detect virus-specific IgA, IgGa, IgGb, IgGc and IgG (T) classes of immunoglobulin in the serum of the inoculated horses. In horse 4 high concentrations of virus-specific IgGa were detected (OD>0.7) twelve days after the second challenge inoculation, and in horse 4 (OD>0.6) eighteen days after inoculation. Only horse 4 exhibited high concentrations of IgGb (OD>0.7), 27 days after inoculation. Very low levels of IgG(T) (OD<0.3) were observed in all horses. No IgGc, and IgA responses (OD<0.2) were observed in any of the horses.

Only horse 4 exhibited high SNT titers (>320), and all the other three horses exhibited very low SNT titers (<20), suggesting that IgGb sub-isotype may be responsible for virus neutralization. These immune responses will be compared to the pattern of cytokine gene expression currently being determined using both qualitative and quantitative RT-PCR.

Effects of anionic salts in a prepartum dairy ration on serum and urine calcium

CJ van Dijk[1], *DC Lourens*[2]

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[2]Department of Production Animal and Community Health.

Manipulating the dietary cation-anion difference (DCAD) of the transition diet of dry cows has been shown to prevent hypocalcaemia and its associated diseases in dairy cows (1,2). The effects of anionic salts in the transition diet on serum and urine calcium levels at calving and on peripartal health, subsequent milk production and fertility performance were studied in a well managed, high producing Friesland dairy herd.

Over a period of a year, approximately 21 days before the expected date of calving, 28 prepartum heifers and 44 multiparous cows were randomly allocated within parity to one of two transition diets designated control and experimental anionic diet. The anionic diet contained the same quantities of the basic transition ration fed to the control group as well as a standard anionic salt mixture containing 118g NH_4Cl, 36g $(NH_4)_2SO_4$ and 68g $MgSO_4$ per animal per day. This reduced the DCAD to −11,680mEq/100g dietary dry matter compared to +13,472 for the control diet. Blood and urine were randomly sampled from 7 animals within each category within 3 hours postpartum. Serum calcium (total and ionised) and creatinine, urine calcium and creatinine and the fractional clearance of calcium were assessed. Relevant clinical, production and fertility data were collected Statistical analyses were done using SAS.

The total serum calcium (2,07 versus 1,60mmol/L), serum ionised calcium (1,12 versus 1,02mmol/L) and fractional clearance of calcium (1,88 versus 0,09 %) were significantly higher ($P<0,01$) at calving for multiparous cows fed the anionic diet compared to those fed the control diet. In the primiparous cows there were no significant differences in serum calcium levels. However, the fractional clearance of calcium was higher (1,75 versus 0,45 %) in primiparous cows fed the anionic diet ($P<0,01$). Therefore, there were benefits, although no differences were demonstrated with respect to health, milk production, or fertility. The low serum calcium level of the multiparous control cows illustrates that many cows experience some degree of hypocalcaemia during the first days after calving. The incidence of milk fever generally increases with parity and with higher levels of milk production and thus higher demands for calcium, regardless of breed.

The mild metabolic acidosis caused by the anionic salts probably increases the receptivity and responsiveness to parathyroid hormone, increases the amount of 1,25 dihydroxy vitamin D, followed by an increase in bone calcium mobilisation and intestinal calcium absorption (1). Further evidence suggests that a better response to a low DCAD diet occurs when the dietary concentration of calcium is increased (2).

Radiography of the thoraco-abdominal cavity of the ostrich (*Struthio camelus*)

WM Wagner, RM Kirberger

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Purpose: Even though ostriches (*Struthio camelus*) are economically important production animals, amazingly little research has been done on them, including the field of diagnostic imaging (1). A few articles have been published on radiography of ratites, but no in depth radiographic study could be found (2). The object of the paper was therefore to establish a standard radiographic procedure for the thoraco-abdominal cavity of female ostriches.

Materials and Methods: A high output rotating anode fixed x-ray apparatus (Polydoros 100 by Siemens), medium speed rare earth screens (Trimax T6) with compatible films and a focused 12:1 grid were used. The source-to-image distance (SID) was kept at 115 cm. One adult ostrich cadaver, two chicks radiographed at about three months intervals from the age of three weeks till 12 months as well as two young adult females (about three years old) were included in the study. All living birds were clinically normal and were allocated to three groups: chicks (less than 16 weeks old); growing birds (older than 16 weeks); and adults (fully grown).

Results: A standard radiographic procedure for the thoraco-abdominal cavity of female non-breeding ostriches including positioning, collimation, centering and a technique chart (3) was produced in order to give reproducible and consistently good quality radiographs. A six-frame technique was established for lateral views taking the topographic tissue distribution into consideration and using easily palpable landmarks as centering points. Standing true right lateral radiographs are recommended for standard procedures. For dorsoventral exposures a three-frame technique in the recumbent ostrich was found to be optimal. Birds should be fasted if possible.

Conclusion: The standardized radiographic technique described provides reasonable to excellent radiographic images of the thoraco-abdominal cavity of varying sized ostriches and possibly other ratites. The technique chart can be adapted to each institution's specific equipment. The technique should result in improved diagnostic capabilities in this species, particularly if combined with a thorough understanding of the radiographic anatomy. Radiography may thus prove to be an equally commonly used imaging technique as in other domestic species.

Hysteroscopy in the bitch

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The aim of this study was to investigate if the endometrium of German shepherd bitches could be examined by means of hysteroscopy. Hysteroscopy could be valuable to diagnose uterine pathology.

Eight post-puberal German shepherd bitches, aged 9-31 months and weighing 25 to 30 kg were used. Two were in dioestrus, 5 in anoestrus and one about 8 weeks post partum. Each bitch was anaesthetised, placed in dorsal recumbency and given 30 µg clenbuterol intravenously. The uterus was exposed through a 4-6 cm ventral celiotomy. A laparoscope, 4 mm with a 30° angle or 3 mm straight, and a catheter for inflation were inserted into the uterus. After hysteroscopy, each bitch was spayed, her uterus opened and examined macroscopically. Samples from each uterine horn and the uterine body, if the latter was removed, were evaluated histologically. Histology was done on normal tissue, lesions that were seen during hysteroscopy, and sites where the endometrium appeared traumatised. Lesions seen during histology were compared with those seen on the video footage of the hysteroscopy. The samples were also evaluated in order to determine the extent of the trauma caused to the uterus during the hysteroscopy.

The endometrium was seen in 6 bitches with the 4 mm laparoscope but, in the other 2, aged 14 and 25 months and in anoestrus, it was too thick and the thinner one had to be used (in one only after the uterus was removed, because the instrument had not been prepared before surgery). The uterotubal junction was seen in all bitches. In 3 bitches the caudal part of the uterine body and the cervix were not seen, because 2 had some uterine discharge and the inflated air escaped through the cervix of the other. In the post-partum bitch dark-brown implantation sites were seen. In 4 young bitches (9, 22, 25 and 31 months old) endometrial cysts between 0.5 and 2 mm in diameter were seen.

Macroscopic examination revealed that hysteroscopy caused acute trauma in the form of petechiae and ecchymoses in the endometrium of 4 bitches. Where the endometrium appeared macroscopically normal, histology revealed that the luminal epithelium was traumatically removed along 0-25% (mean 4%) of the tissue length, compared to 0-95% (mean 33%) in sections from areas where signs of trauma were seen macroscopically. The glandular crypts were intact along the entire length of all sections.

The angulated laparoscope is preferred, because the uterine wall can be inspected in more detail, which is essential to see small lesions.

Hysteroscopy is a very sensitive tool to evaluate the macroscopic appearance of the canine endometrium. Because the glandular crypts were not destroyed it is likely that the epithelial trauma would be repaired within 3 to 5 weeks as during any normal anoestrous period, although further research is necessary to determine the effect of the procedure upon future fertility.

Liver perfusion with hyperosmotic media stimulates glutamine and urea synthesis

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Introduction

Ammonia is detoxified in the liver by both the liver-specific pathway of urea synthesis and by glutamine synthesis. Most of the physiological portal ammonia load is converted to urea by periportal hepatocytes (near the sinusoidal inflow) while the remainder is converted to glutamine by the perivenous hepatocytes (near the sinusoidal outflow). Glutamine synthesis removes ammonia that bypasses the urea cycle, thus preventing hyperammonia in the peripheral circulation. In rats, numerous investigations have shown that pH and hormones modify hepatic synthesis of both glutamine and urea. Although we have previously demonstrated that pH does not modify ammonia partitioning between urea and glutamine synthesis in the ovine liver, recent evidence suggests that alterations of cell volume modulate hepatic metabolism. In this study, we examined the role of cell shrinkage in ureagenesis and glutamine release in the perfused caudal lobe of sheep liver.

Materials and Methods

Twelve wethers weighing 23-33 kg liveweight were fed *ad libitum* a mixed diet of lucerne and tef hay. The water intake of these sheep was restricted for four days to 50 % before isolating the caudal lobe of the liver. Isolated lobes were perfused as described previously (Ali *et al.* 2000) in a non-recirculating manner with Krebs-Henseleit buffer containing 0.5 mM NH_4Cl. The osmolarity of the buffer (300 mOsmol/kg H_2O, control) was adjusted to 330 mOsmol/kg using either NaCl or sucrose (hyperosmotic media). The effluent sample was collected at a regular interval throughout the perfusion. The concentrations of ammonia, urea and glutamine were determined in the influent and effluent perfusate.

Results and Discussion

The results are shown in Fig.1. Although increasing the osmolarity of the medium did not affect ammonia uptake by the liver, those exposed to the NaCl medium tended to take up more than control livers. Perfusion with hyperosmotic medium increased urea production by 10-23 %. Similarly, hyperosmotic exposure increased glutamine release by 100-170 %. The effect of cell shrinkage on glutamine release was by far the most pronounced, where both hyperosmotic media significantly increased this parameter.

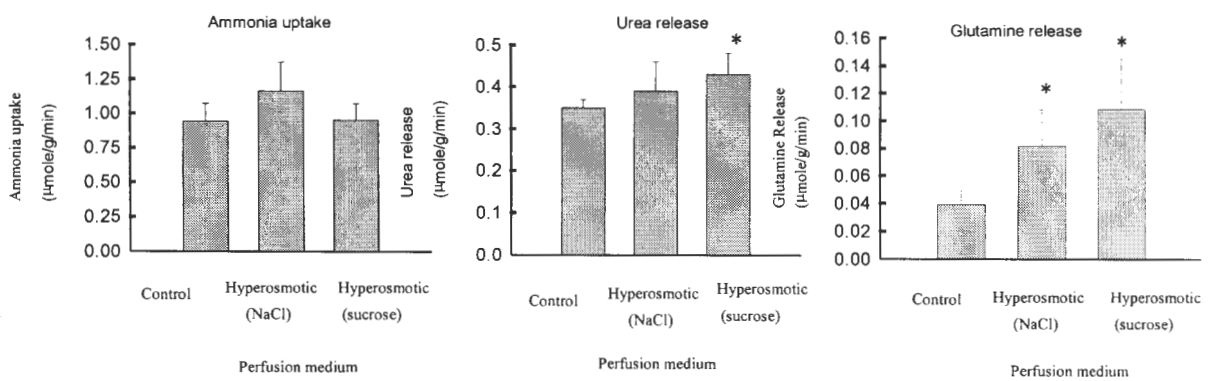

Fig.1. Effect of cell shrinkage on nitrogen metabolism in perfused sheep liver.

* Mean value significantly different from control value ($P < 0.05$)

This increase in urea synthesis and glutamine release can be explained by an increase in proteolysis. Cell shrinkage acts as a catabolic signal, leading, for example, to stimulation of proteolysis. In the rat, it has been shown that cell shrinkage triggered by exposure to hyperosmotic medium or by hormones such as glucagon, increases glutamine synthesis and urea synthesis (Haussinger *et al.* 1994). In conclusion, our results suggest a prime role for cell volume in the regulation of nitrogen metabolism in sheep liver.

Vector competence of selected South African *Culicoides* species for the Bryanston serotype of equine encephalosis virus

GJ Venter[1], *DM Groenewald*[2], *JT Paweska*[1], *EH Venter*[2], *PG Howell*[2]

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[2]Department of Veterinary Tropical Diseases

Equine encephalosis virus (EEV) was recognized and described in the Republic of South Africa in 1967 and subsequent serological studies have shown this orbivirus to be both widespread and prevalent in southern Africa.

In the present study it was shown that wild-caught *Culicoides (Avaritia) imicola* can become infected with and permit the replication of the Bryanston serotype of EEV following membrane-feeding on infective blood containing 5.0 $\log_{10}$ plaque-forming-units (PFU)/ml.

Prevalence of virus infection in *C. imicola* was determined by PFU and microtitration assays on both BHK- and Vero cells and confirmation of the Bryanston serotype of EEV was determined by plaque inhibition.

The mean prevalence of Bryanston virus infection in *C. imicola* after 10 days extrinsic incubation at 23.5 °C was 22.3% (23/103). The mean infectivity of Bryanston virus in the infected *C. imicola* increased from 1.3 $\log_{10}$ PFU/midge, in insects assayed immediately after feeding on the blood-virus mixture, to 2.6 $\log_{10}$ PFU/midge in insects assayed after incubation. The virus concentration in individual *C. imicola* infected with the Bryanston serotype of EEV ranged from 0.7-3.6 $\log_{10}$ PFU/midge.

Bryanston virus titres higher than 2.5 $\log_{10}TCID_{50}$, found in individual *C. imicola*, suggest that this species may be able to transmit this virus to susceptible hosts. No virus replication could be demonstrated in 102 *C. nivosus* tested after the incubation period, suggesting that not all *Culicoides* species are equally susceptible to Bryanston virus infection.

Currently these techniques are used to test other *Culicoides* species as potential vectors for EEV.

The use of community action plans to facilitate grassroots development of animal health and production programmes

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[2]Community Development Academy, University of Missouri, Columbia, Missouri, USA.

Agriculture is a complex social process, not simply a technical activity. As the paradigm shifts from delivery of social services, including the transfer of technology, to the empowerment of people, the challenge is to develop the capacity of communities to allow them to be involved in decisions that affect their future. They need to learn to use their own assets to leverage other resources and pursue their own interests with local initiatives[1].

The use of participatory methods is important for sustainable development. People need to be involved in meaningful ways if successful development is to occur. Community participation can take many different forms ranging from passive participation through to interactive participation and self-mobilisation[2]. Benefits are more likely to be sustainable when participation has reached the interactive and self-mobilisation stage.

Certain principles are important in the use of participatory methods[3]. These include a focus on cumulative learning in participative ways to seek diversity. Group inquiry is more likely to reveal the complexity of the farming system. Actions should be flexible in order to suit each new set of conditions. The 'expert' acts as a facilitator to bring about changes which people in the situation regard as improvements. The process is about debate and change followed by reflection. Action to take is agreed upon which accommodates different conflicting views and leads to more sustainable actions.

During the development of animal health and production programmes for communal farmers in the Madinyane area an action planning process was used to build social capital and to move away from a dependency syndrome. Preliminary planning sessions were followed by community workshops where a broad vision was created. Participants then developed their own projects in the field of animal health and production. The implementation phase requires the formulation of detailed objectives and facilitation of what people need to do, so that farmers can take leadership of the programme.

Anaesthetic sparing effect of midazolam in propofol induced, and isoflurane maintained anaesthesia during ovariohysterectomy in dogs

GF Stegmann, L Bester Department of Companion Animal Surgery, University of Pretoria, South Africa

The purpose of this investigation was to determine the influence of midazolam premedication on the dose required for the induction and maintainenance of surgical anaesthesia in dogs with propofol and isoflurane respectively.

Ten German Shepherd bitches, scheduled for ovariohysterectomy, with a body weight between 25 and 30 kg, and nine to 31 months old, were randomly allocated to two groups. Group A was premedicated two minutes before induction with IV midazolam at a dose of 0.2 mg/kg. Group B was treated with saline placebo. Both groups were induced with IV propofol (Diprivan, Zeneca) at 4 mg/kg as a bolus over 60 sec. Incremental propofol (1 mg/kg) was added until the pedal reflex was absent. Anaesthesia was maintained with a circle anaesthetic machine with an out-of-circuit precision isoflurane vaporizer (Fortec, Cyprane). End-tidal isoflurane concentration (ETiso) was monitored with an inhalation agent analyzer (Capnomac Ultima, Datex). Data were reported as mean (±SD). Two-way repeated measures ANOVA was used to analyze the data for difference in treatment. The Students' t-test was used to test for differences in propofol doses. Significance was accepted at $P< 0.05$. The investigation was approved by the Ethics and Research Committees of the University of Pretoria.

The mean incremental propofol dose required to obtain loss of the pedal reflex was 3.4 ± 0.8 mg/kg for Group A, and 5.4 ± 0.8 mg/kg for Group B. The difference between the two groups was statistically significant ($P<0.05$). The ETiso for Group A and B to maintain loss of the pedal reflex during surgery was 1.7 ± 0.3 and 2.2 ± 0.5 % respectively. The difference between the two groups appeared to be statistically significant ($P<0.05$).

The propofol dose required to induce anaesthesia in man were reduced by 52 % (1). In this investigation a 37 % reduction in the anaesthetic dose for propofol was obtained. Midazolam premedication resulted in the ETiso for surgical anaesthesia to be reduced by 23 %. The MAC for isoflurane in dogs is 1.28 % (2), and this represented a decrease from 1.72xMAC to 1.33xMAC in dogs after midazolam premedication. The intensity of noxious stimuli may vary during surgery, and this may have contributed to the variability in the ETiso values that were obtained during this investigation. It is concluded that midazolam premedication in dogs significantly reduce the dose required for surgical anaesthesia after propofol induction, and a tendency to reduce the isoflurane concentration during maintenance of anaesthesia.

Evaluation of the diagnostic usefulness of serum uric acid concentration in the assessment of liver function

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In order to determine whether liver disease is present in canine patients, it is customary to evaluate the serum activity of enzymes (like ALT), which are typical of liver tissue. These will be elevated if there has been recent degeneration or necrosis of hepatocytes. This information is useful but reveals nothing about the extent of liver function compromise. It has become generally accepted that serum bile acids and plasma ammonia are the best tests of liver function. These tests are expensive. Additionally, bile acid assay is only available from a half dozen veterinary laboratories in South Africa where they "batch" the requests to reduce costs (but prolong waiting time) and ammonia is very unstable in *vitro* making it is a test that practitioners cannot request due to delays in getting samples to a laboratory.

Uric acid is an oxidation product of purine metabolism in vertebrates. In all mammals, except humans and some other primates, uric acid is taken up by hepatocytes and converted, by the enzyme uricase to allantoin, which is easily excreted by renal filtration. In these species, serum uric acid concentrations only rise to the level encountered in humans when there is a significant reduction in functional hepatic mass. Among domestic dog species, Dalmations represent a rare exception due to an ineffective hepatic cell membrane transport mechanism which causes them to have higher serum uric acid concentration. During the Fifties, serum uric acid developed a reputation as a reliable index of liver function. This reputation was dealt a blow by three publications, two in 1959 and one in 1961, which upon review, were seriously flawed. These publications have been cited ever-since as reason for not using serum uric acid as a liver function test in dogs. Due to its usefulness as a test of the presence of or predilection to gout, in man, the test is available in all laboratories throughout the country. The reagent costs per test are less than 10% of those of serum bile acids and plasma ammonia.

A retrospective "pilot trial" was conducted on 93 of 450 sera from dogs on which either serum bile acids and plasma ammonia had been requested previously. These sera were assayed for uric acid. The classification consistency for the diagnosis of "liver dysfunction" was compared, as were the diagnostic indices.

Classification consistency was found to exceed 75% for both serum uric acid *versus* serum bile acids as well as plasma ammonia. Serum uric acid had a sensitivity in excess of 75% and a specificity of 90% for the diagnosis of "liver dysfunction". These indices compared very favourably with those for serum bile acids as well as plasma ammonia. That, subject to the confirmation of these findings by a prospective study, where the diagnosis of hepatic disease is confirmed by biopsy, veterinarians will be able to assess liver dysfunction in a large proportion of their canine patients by ordering serum uric acid as an initial screening test at some 10 to 20% of the cost of the more "famous" tests.

Pharmacological enhancement of emission and ejaculation in cattle: a look at imipramine hydrochloride

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Seminal emission appears to be controlled by α-adrenergic mechanisms. Imipramine hydrochloride (IMI), is a synthetically manufactured tricyclic antidepressant (TCA). Tricyclic antidepressants potentiate the actions of biogenic amines (noradrenaline, serotonin and dopamine) centrally and peripherally, by blocking their re-uptake into nerve terminals. In this manner they have an adrenergic potentiating effect. This effect is anticipated to be of clinical use in semen collection in bulls. In stallions imipramine is used to facilitate emission and ejaculation and concurrently increasing the sperm concentration in the ejaculate. The aim of this study was to determine the pharmacokinetics of imipramine in bulls and whether it can be used to alter the contractility of the smooth muscles of the ampullae *in vitro*.

To establish the pharmacokinetics of IMI in bulls, imipramine hydrochloride (Centre for Pharmaceutical Services, Technikon Pretoria, South Africa) was administered intravenously to three bulls (600 - 705.5 kg) at a dose of 2mg/kg of body weight (bw). Intravenous plasma concentrations of imipramine were determined by fluorescence polarisation immunoassay (FPIA). A two compartmental open model with first-order rate constants best described the imipramine plasma concentration versus time profile. Imipramine distributed rapidly, ($t_{1/2\alpha}$) of 7.2 ± 4.2 min, exhibited a very large apparent steady state volume of distribution (Vd_{ss}) of 4.2 ± 0.9 ℓ/kg body weight, had a very short terminal elimination half-life ($t_{1/2\beta}$) of 140 ± 15 min and showed a rapid total body clearance (Cℓ) of 22.7 ± 7 mℓ/min/kg. In all animals, treatment with IMI resulted in pronounced central nervous system signs immediately post injection. All CNS signs dissipated 15—20 minutes post injection. An interval of 23 hours between repeat treatments of IMI was established. Spontaneous emission and ejaculation was not observed in cattle at this dose. Studies supporting adrenergic potentiating effects of imipramine, report such effects at doses of ≤1 mg/kg bw, with higher doses having paradoxical effects. Thus, lower doses should be used when emission of a concentrated semen sample is the objective. Use of IMI in bulls for semen collection purposes still needs to be investigated with regard to dose and time in order to achieve an optimal effect on emission and ejaculation.

The ampullae of bulls were stimulated with noradrenaline (NOR) and IMI *in vitro*. Noradrenaline alone consistently produced dose dependant smooth muscle contractions of the ampulla. Imipramine alone had no effect on the contractility of the smooth muscles. In combination with NOR, IMI altered the smooth muscle contractility. The frequency of the contractions was decreased while the amplitude was increased.

The results of this study indicate that imipramine may be useful to increase the number of ejaculated sperm if semen is collected from bulls by electroejaculation. An *in vivo* study is necessary to prove this possible benefit of imipramine effect.

Fertility of bitches after intravaginal insemination with frozen-thawed sperm extended with either prostatic fluid or protein-free TALP prior to insemination.

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Bitches that were inseminated into the vagina with frozen-thawed semen to which 7 ml prostatic fluid was added prior to insemination had higher fecundity than bitches that received similar semen but no additional fluid (1). It is unknown whether the effect of prostatic fluid was merely due to increased volume of the inseminate or not. The aim of this study was to determine whether the fecundity of bitches would be higher if they are inseminated with frozen-thawed dog semen to which 7 ml prostatic fluid, instead of 7 ml TALP, which like prostatic fluid has a watery consistency but maintains the *in-vitro* motility of frozen-thawed dog sperm better, was added to it.

German shepherd bitches (n=28) were inseminated into the fornix of the vagina with semen from two donors. All semen was frozen and thawed in the same way. For the 15 Group P bitches and the 13 Group T bitches, sperm-free, frozen-thawed prostatic fluid or protein-free TALP were, respectively, added to the thawed semen. Bitches were inseminated daily with 50×10^6 progressively motile sperm while the vaginal folds were angular and spayed 19 d after the last insemination. Implantation Rate (IR) was defined as the ratio between the number of implanted conceptuses and the number of *corpora lutea* in each bitch and litter size (LS) as the number of viable conceptuses in pregnant bitches.

The data of three bitches, of which none conceived, were removed from further analysis: One Group T bitch accidentally received only 30 million progressively motile sperm on some days, whereas one Goup P bitch became ill during late oestrus due to babesiosis and another Group P bitch had a blocked uterine tube ipselateral to the ovary that contained eight corpora lutea, while her other ovary had only one *corpus luteum*.

Pregnancy rate was 77% for Group P (n=13) and 83% (n=12) for Group T ($P>0.05$). Mean litter size was 7.2 (SD 3.8, n=10) for Group P compared to 4.1 (SD 3.2, n=9) for Group T ($P<0.05$), whereas the the number of *corpora lutea* did not differ (the median, 25th and 75th percentiles were 11, 10, 11 and 10, 9 and 11, respectively, P=0.4). The mean implantation rate was 0.55 (SD 0.42) for all 13 Group P bitches, which was not higher than the 0.38 (SD 0.36) for all 12 that received modified TALP (P=0.15, power=0.12 with $\alpha=0.1$).

This study shows that the effect of prostatic fluid on the fertility of vaginally inseminated frozen-thawed dog semen is not due to changing the volume or viscosity of the inseminate.

Ultrasound-guided injection into the *crus penis* for cavernosography in bulls

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Blockage of the dorsal canal of the *corpus cavernosum penis* is a cause of erection failure in bulls. The only means of confirming this condition antemortally is the surgical exposure of and injection of contrast medium into the penile crurae followed by radiographs of the distal penis to detect through flow. The aim of this study was to develop a non-surgical technique for injecting contrast medium into the *crus penis* and to evaluate the ability to demonstrate contrast medium in the distal portion of the penis in normal bulls.

Five healthy adult bulls of three different breeds, all with normal serving ability, were used, with one being subjected to the procedure twice. Each bull was starved, general anaesthesia was induced with xylazine (Rompun, Bayer) and ketamine (Anaket V, Centaur) and maintained with halothane (Fluothane, Zeneca). With the bull in lateral recumbency the penis was extended and two perspex bars were sutured together, one on either side of the preputium dorsal to the penis, holding the penis away from the abdomen to permit radiography of this portion of the penis. Survey radiographs were taken to ensure suitable exposure. An Aloka 500V ultrasound scanner with a 5-MHz convex linear transducer was used to identify the *crus penis*. An 18 gauge spinal needle was passed through a stab incision in the skin and into the *crus penis* under ultrasound guidance and two syringes containing an iodine-based contrast medium (Conray 420, Rhône-Poulenc Rorer) were connected to it via a three-way stopcock. Stimulation using an electro-ejaculator with a rectal probe was initiated. When the penis started developing an erection, 50 to 100 ml of contrast medium was injected as rapidly as possible. Lateral and ventrodorsal radiographs were taken of the extended penis during and at intervals after injection. After a rest period of 5 minutes, a radiograph was taken to ensure clearance of the contrast medium and the procedure was repeated on the *crus penis* on the other side.

Successful cannulation of the *crus penis* was confirmed by visible indentation of the fibrous capsule by the needle, free flow of blood on entering the *crus*, lack of resistance to the injection of air, which could clearly be seen in the *crus*, and the fluctuation of resistance to injection in synchrony with the pulsation of the electro-ejaculator. Contrast medium was demonstrated in the distal penis in all six cases, or on 8 of the 12 attempts. Factors, which enhanced through flow of contrast medium, were attainment of penile erection, a larger volume of contrast medium, and the choice of upper or lower *crus penis*, with the uppermost being easier to cannulate. On one occasion the needle worked out of the *crus penis* during stimulation, which resulted in injection of contrast medium into the *corpus spongiosum penis*. All bulls recovered uneventfully and returned to normal serving ability after the procedure.

Cyclical morphological changes in the efferent ducts of birds

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Discrepancies in the reported structure of the various ducts of the avian epididymis[1,2], are probably due to the varying stages in the reproductive cycle of the birds studied. This paper evaluates the morphology of the efferent ducts during the reproductive cycle (prepuberal, sexually mature and active, and sexually mature but inactive stages) of the domestic fowl (*Gallus domesticus*), drake (*Anas platyrhynchos*) and guinea fowl (*Numida meleagris*).

The birds studied were: (1) *Prepuberal*, 5 10-wk-old domestic fowls (DF); (2) *sexually mature and active*, 5 drake (DR), 5 DF and 3 guinea fowls (GF); (3) *sexually mature but inactive*, 3 oestrogen-treated domestic fowl (given a single dose of 50 mg oestradiol benzoate SC for 5 d), 5 DR and 5 GR. The epididymides of these birds were prepared for electronmicroscopy[1], and the efferent ducts (proximal, PED and distal, DED) were studied.

The terminology of Aire[1] is adopted. In prepuberal DF, the non-ciliated, Types I and II, cells of the PED and DED, respectively, displayed oval and only marginally heterochromatic nuclei. A few round homogeneous dense bodies and undeveloped subapical tubulo-vesicular system were seen in the Type I cells. Mitochondria were larger in the non-ciliated than ciliated cells. Luminal macrophages were present in the PED. In sexually active birds, the nuclei of the Types I and II cells were regularly oval in shape. Unlike in the prepuberal DF, the dense bodies in the Type I cells were numerous and often heterogeneous. The subapical tubulo-vesicular system was elaborate and conspicuous.

In the sexually inactive birds, there were luminal macrophages in the PED, as in the prepuberal birds. The subapical tubulo-vesicular system in the Type I cells had atrophied. Numerous, large lipid droplets occurred, mostly basally, and dense bodies were very large, usually irregular in shape and heterogeneous. Nuclei were irregularly-shaped and more heterochromatic in all cell types than in prepuberal and sexually active birds. The mitochondria of the non-ciliated cells were similar to or smaller than those of the ciliated cells, in size. The ground substance of the cytoplasm was slightly rarefied.

The varying organelle content, conformation and disposition in the three epithelial cell types of the efferent ducts seem to be phase-specific, and may therefore, along with testicular changes[3], form a basis for the determination of the phase of the reproductive cycle in a male bird, especially in seasonal breeding species.

Molecular epidemiology of serotype O foot-and-mouth disease virus in West Africa (1988-1993)

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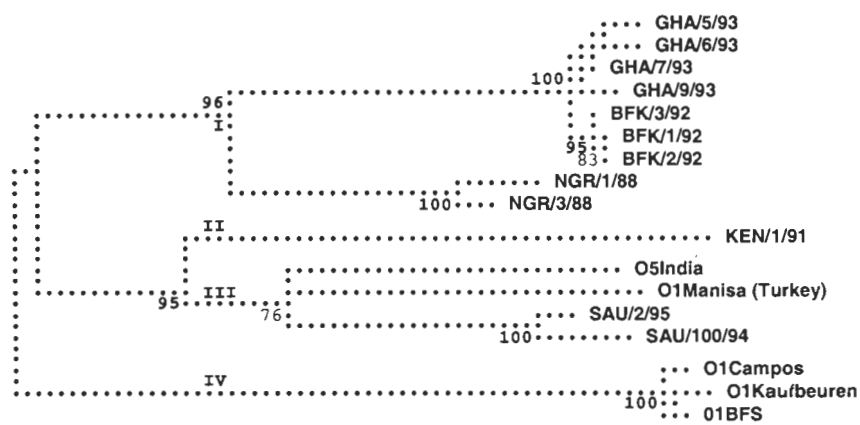
Genetic relationships of nine serotype O foot-and-mouth disease (FMD) viruses recovered from outbreaks of the disease in Niger, Burkino Faso and Ghana (1988-1993) were determined by partial VP1 gene characterization. Viral RNA was reverse tanscribed and a fragment of approximate 550 bp was amplified using the polymerase chain reaction (PCR).

An homologous region of 392 nucleotides was aligned, translated and compared to serotype O viruses from the Middle East, Asia, Europe and South America. Four distinct genetic lineages could be identified by phylogenetic analysis. These lineages comprised viruses of the the following geographical origin: Lineage I: West Africa, Lineage II: East Africa, Lineage III: Asia and the Middle East and Lineage IV: Europe and South America. The data further indicates that there are two distinct West African virus genotypes and that the outbreaks in Burkina Faso (1992) and Ghana (1993) are part of the same epizootic. Overall variation for the West African lineage was 13 % and 10 % on nucleotide and amino acid levels respectively.



Scale: each ..... is approximately equal to the distance of 1.8 %

Housing of goats by small-scale farmers

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Goat farmers lose more than 30% of their young stock every year, particularly during the rainy season. This reduces productivity and profit for small-scale goat farmers. One factor contributing to this loss is probably poorly built and unhygienic housing.
The first duty of a goat house is to provide shelter. Goats need housing that is dry, well ventilated and free of draughts. Young goats are very susceptible to the effect of cold and wet and can become so stressed that they die. In addition, sufficient floor space is needed, particularly when a goat doe is kidding. Otherwise there is a chance that the mother will not bond with the new-born kid. The housing should also provide protection from predators.
Research was conducted in Jericho, a rural area in Northwest Province and 15 herds of goats were studied. The methods used for the evaluation of housing were a housing checklist and photographic recording. The overcrowding co-efficient (the number of animals divided by the square meters of the floor of the house), overhead shelter, shelter from the effects of draughts, ventilation, drainage and hygienic conditions of the housing were evaluated. Space per animal is fairly critical with a minimum of 0.8 square meters per adult animal. Hygiene was measured by asking the farmers how often they removed faeces from the kraal. Goat houses at this area are made of wire, scrap and corrugated iron, thorn bushes and wooden poles. Bad roofing is common. This results in leakage of water during the rainy season and floors become muddy. It was found that 40% of the houses provide no shelter from rain and the remaining 60% provide partial shelter from rain.
In terms of shelter from the prevailing wind it was found that 47% of the houses could not provide shelter from the prevailing wind and 53% could provide shelter from the prevailing wind. Only one house had insufficient ventilation, as the owner was worried about stock-theft. One farmer had five kids killed by a dog. Drainage was measured by using a score of 1-5 where a score of one was regarded as very poor drainage and 5 as very good drainage. In this case it was found that 27% of houses had poor drainage and 73% of the houses had moderate drainage. It was found that 98% of the respondents did not remove faeces from the floor and 2% of the respondents removed faeces from the floors twice to three times a year. In terms of overcrowding, it was found that 60% of the houses were not overcrowded (overcrowding co-efficient < 0.8 sq m/adult goat) and 40% of the houses were overcrowded (overcrowding co-efficient of > 0.8 sq m/adult goat).
It is probable that the wet conditions, lack of shelter from the prevailing wind and poor drainage contribute to the mortality of kids due to cold stress. Kids that died just after birth could be as a result of overcrowding, however there did not appear to be a correlation between this kind of mortality and the overcrowding coefficient in the herds studied. Lack of hygiene and build up of faeces could result in the kids becoming infected with bacterial diseases and internal parasites. It is clear from the initial findings of the survey of housing that extension is required to improve the housing of goats in the area studied.

Protection of vaccinated goats against experimental challenge with *Mannheimia haemolytica* a 1 leukotoxin

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Mannheimia haemolytica is the cause of serofibrinous pleuropneumonia in goats and sheep in South Africa (3). It has been established that the leukotoxin, which is an extracellular toxin produced during active growth of the organism, is one of the major components responsible for the pathogenesis of this disease (2). A vaccine was produced that contained leukotoxin as the main protective antigen and administered to goats which were challenged intratrachealy with live *M. haemolytica* type 1 cultures.

The objectives of this trial was to test the efficacy of the *M haemolytica* leukotoxin vaccine in goats and to establish an effective challenge model by means of a clinical scoring system. The leukotoxin was produced in RPMI 1640 medium culture supernatant after 3 hours active growth with a high leukotoxin producing type 1 strain of *M. haemolytica*. The leukotoxin content was evaluated by means of an antigen ELISA test (1) and the culture supernatant was subsequently harvested by centrifugation and filtration and finally dispersed in 10 mL plastic vaccine containers and kept at 4 to 6 ^{0}C in a refrigerator. Four male Saanen goats, 1 year old were used as experimental animals and vaccinated twice with 1 mL of the leukotoxin vaccine subcutaneously 14 days apart. Five Saanen goats of the same age were used as negative controls and were not vaccinated at all and only received a placebo of normal sterile physiological saline. All the goats were challenged with 10 mL of active culture containing 1 x 109 cfu per mL *M. haemolytica* intratrachealy with a sterile Bardicath intravenous placement unit 7 days after the second vaccination. For 5 days post inoculation the goats were observed for the following clinical parameters: coughing, ocular/nasal discharge, dyspnoea, inappetance, lethargy and recumbency. A scoring system was devised and used to quantitate the clinical signs and scores were accumulated for each day after the challenge was administered. For each day one point was allocated to a specific clinical manifestation if it was positive and none if it was negative. If the animal died it was given a score of 6 for that day Finally the score was added for each animal in the experimental and control group and compared for each day.

The second day three of the five control animals died and the remaining two scored 5 out of a possible six. On days 3 and 4 the condition of these 2 animals remained the same and it was decided to euthanase them. Autopsies were performed on each animal and lung samples collected for histopathology and bacterial isolation and typing of the organism. None of the four animals in the experimental group manifested any symptoms and had no scores for the five day observation period. The results from the control animals confirmed the mortality was due to the experimental infection with *M. haemolytica*. In conclusion it may be stated that the *M. haemolytica* leukotoxin vaccine is protective in vaccinated goats after challenge with virulent *M. haemolytica* organisms.

Canine DNA Testing

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Although Canine genotyping should be conducted on a routine basis as an integral part of the registration process to ensure the accuracy of the pedigree it is presently only done on an "as needed basis". The need exist to resolve questionable breeding situations such as multiple-sired litters, puppies mixed up between litters, intentional substitutions of a sire, stolen dogs, and to prove parentage to identify a dog. Such problems may involve closely related dogs and to resolve such cases the markers must be extremely informative.

Blood collected in EDTA is used. Buffy coats are used for PCR amplification and the samples are prepared for running on the 310 Genetic Analyzer according to the protocol. A panel of 10 highly polymorphic short tandem repeat DNA markers, directed at 9 tetranucleotide and 1 trinucleotide repeat sequences that provides standardized date for all canine genotyping, are tested. Each of the loci have been evaluated for Mendelian inheritance over multiple generations.

Case studies to prove parentage is illustrated showing a primary battery of 10 STR loci provides rigorous parentage analysis and individual identification.

Comparison between two trans-vaginal oocyte recovery sessions three days apart in African Buffalo (*Syncerus caffer*)

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The quality of bovine oocytes improves when ultrasound guided trans vaginal oocyte recoveries (TVR) are repeated with 3-4 day intervals (1). The aim of this study was to test if the number and quality of oocytes recovered by TVR during tuberculosis testing differs between the two sessions.

Thirteen adult buffalo cows were used in the study. They were immobilised twice, 3 days apart, with M99. TVR on both occasions was performed in each cow as described earlier (2). The number of follicles aspirated, the number of oocytes retrieved and their quality were recorded according to the following scale: grade 1=complete cumulus of several layers; grade 2=complete cumulus; grade 3=few cumulus cells and evenly granulated cytoplasm; 4=few or no cumulus cells and unevenly granulated cytoplasm; expanded=any amount of expanded cumulus or granulosa cells; degenerated=empty zonas, non-spherical or obviously degenerating eggs. The aspiration results were compared with the paired *t*-test (P 0.05).

Table 1: Follicles aspirated and oocytes retrieved during two TVR sessions in 13 African buffalo.

day	Mean number of follicles per cow	Mean number of oocytes per cow	recovery rate (%)
1	6.2 (range 3-10)	2.7 (range 0-9)	45.7
4	4.8 (range 3-7)	2.5 (range 0-5)	46.4

Table 2: Number of eggs retrieved during two TVR sessions in 13 African buffalo.

day	grade 1	grade 2	grade 3	grade 4	expanded	degenerate
1	1 (2.7%)	6 (16.2%)	17 (45.9%)	8 (21.6%)	1 (2.7%)	4 (10.8%)
4	1 (3.1%)	6 (18.8%)	9 (28.1%)	9 (28.1%)	3 (9.4%)	4 (12.5%)

There was no difference between the 2 aspirations in terms of any of the variables and there was no correlation between the number of follicles seen on the 2 sessions (Pearson's correlation coefficient 0.05; P 0.9). Two aspirations do not appear to be sufficient to synchronise the follicular waves and to improve the number and quality of oocytes recovered, as described in cattle after multiple aspirations (1).

Development of morula stage embryos after trans-vaginal oocyte recovery of the African buffalo, *Syncerus caffer*

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The genetic diversity of the disease-free African buffalo population, *Syncerus caffer*, is limited. With the ultimate objective of improved gene flow from the larger diseased populations to the smaller disease-free population, *in vitro* embryo technologies were utilised. Oocytes were collected from immobilised cows by means of trans vaginal ultrasound guided aspiration, matured, fertilised and cultured *in vitro*.

Buffalo cows housed in the Phinda Nature Resource, KwaZulu Natal, RSA, were immobilised using etorphine. Oocytes were collected on two occasions from each recumbent cow using an Aloka SSD-500 ultrasound machine and a 5 MHz convex ultrasound probe (Aloka, UST-974-5). All oocyte and embryo culture was performed in petri dishes containing 50 µl droplets under oil, sealed in gas-tight plastic bags filled with humidified 5 % CO_2 in air and submerged in a 39°C water bath. Recovered oocytes were matured in TCM 199 supplemented with 25 µg/ml gentamicin, 100 µM 2-mercaptoethylamine, 25 mM HEPES and 10 % *Bos taurus* steer serum for 22 h. After maturation, sperm previously frozen from 4 buffalo bulls was thawed, mixed, swim-up separated and added to oocytes at a final concentration of $2x10^6$ sperm/ml in a modified TALP media including 25 µg/ml gentamicin, 30 µg/ml heparin and PHE (2.0 mM penicillamine, 1.0 mM hypotaurine and 25 µM epinephrine). Oocytes aspirated during the first collections were fertilised in the presence of 10 % steer serum. In the second 0.6 % FAF BSA was used. Oocytes with expanded cumulus cells at the time of aspiration were matured for 4 hours and then fertilised. Oocytes which were obviously degenerating were not cultured. During the fertilisation period of the oocytes after the second aspirations, it was discovered that the electricity had cut out overnight and the water bath had reached a temperature of 25°C by morning. The water temperature was brought back quickly up to 39 °C. Upon recovery of temperature, the oocytes were stripped of cumulus cells and sperm and placed into co-culture droplets with buffalo granulosa cells in TCM 199 with 10 % steer serum for a further 7 days. Cleaved oocytes were counted approximately 48 h (2 days) after the addition of sperm, while embryo development was evaluated 7 days after the addition of sperm.

Fertlised in:	Hours of maturation	No. of oocytes	No. cleaved	No. morulae
10 % steer serum	4	5	0	0
	22	15	0	0
0.6 % BSA	4	2	1 (50%)	0
	22	21	7 (33%)	3 (14%)

No cleavage occurred in the first group of 20 oocytes, fertilised in the presence of 10 % steer serum. However, in the second group, fertilised in 0.6 % BSA, 8 of the 23 oocytes chosen for fertilisation cleaved to at least two cells, despite the temperature drop to 25°C during fertilisation (or culture for the 44-hour matured eggs) period. Three embryos reached the morula stage by day 7. The results using BSA for fertilisation are similar to others reporting on culture of oocytes recovered from culled buffalo.

Follicle numbers and oocyte recovery from ovaries of culled Africa buffalo, *Syncerus caffer*

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The genetic diversity of disease-free African buffalo, *Syncerus caffer*, is limited. Improved gene flow from the larger diseased populations (carrying tuberculosis, corridor disease and/or foot and mouth disease) to the smaller disease-free population is possible using embryo technologies. With this objective in mind, the number and quality of follicles and oocytes on ovaries from culled buffalo was investigated.

In October 1998, buffalo with tuberculosis in the Umfolozi Game Reserve, KwaZulu Natal, RSA were culled using scoline. The animals were divided into 3 rough categories: J, juvenile animals (n=15); A, adults that were either not pregnant (n=3) or in early pregnancy (n=19); and LP, animals near the end of pregnancy (n=6). Ovaries were collected within 3 hours of slaughter and were transported at approximately 28°C to the field laboratory within 1-5 hours. Follicles were counted and placed into 3 categories by size: <3mm, 3-7 mm, and >7mm. Oocytes were retrieved from the follicles by lightly slicing the surface of the ovaries into TCM199 + 10% fetal calf serum with a razor blade. Results were analysed using the Mann Whitney Rank Sum Test.

The number of follicles present and oocytes recovered from ovaries of juvenile (J), adult non- or early-pregnant (A), or late-pregnant (LP) African buffalo are presented in the table below+.

		Follicles per ovary[1]				Oocytes per ovary[1]
	n	<3mm	3-7mm	>7mm	Total	
J	30	14.8 ± 2.9^{c}	1.7 ± 0.4^{c}	0.7 ± 0.2^{a}	17.2 ± 2.9^{a}	8.1 ± 1.8
A	44	12.2 ± 1.6^{c}	2.9 ± 0.5^{c}	1.0 ± 0.3^{a}	16.1 ± 1.4^{a}	9.0 ± 2.6
LP	12	6.0 ± 1.2^{d}	0.8 ± 0.3^{d}	0^{b}	6.8 ± 1.3^{b}	5.2 ± 5.8

[1] mean ± SEM; within columns ^{a,b} indicates rows significantly different ($p<0.005$), while ^{c,d} indicates rows with a tendency to be different ($p<0.10$)

Buffalo in late pregnancy had significantly less follicles (6.8 ± 1.3) than the other two groups (16.1 ± 1.4 and 17.2 ± 2.9 for the A-group and J-group respectively). They had significantly fewer large follicles, and a tendency to fewer medium and small sized follicles. No animals in the J category were pregnant or had corpora lutea.

Most of the follicles present on the buffalo ovaries were very small (<1mm), resulting in a low number of good quality oocytes recovered despite a relatively high number of total follicles. It appears, however, that more oocytes of better quality may be recovered from African buffalo than from some other non-domestic bovine species.

A new disposable needle guide system for trans vaginal oocyte recovery (TVR) in cows and African buffalo (Syncerus caffer)

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Over the last decade, many different systems have been developed to utilise ultrasound guidance for oocyte retrieval from living cattle. Most systems available make use of long, non-disposable needles. These needles have several disadvantages over disposable needles: they are expensive, they get blunt after a few aspiration sessions and even after resharpening they are not as sharp as new needles. A further disadvantage of many ultrasound probe handles is their tendency to slip out of the vagina unless constantly held in place. The aim of this study was to design an ultrasound needle guide for use with disposable needles that would not easily slip out of the vagina.

An Aloka SSD-500 ultrasound machine with a 5 MHz convex ultrasound probe (Aloka, UST-974-5) was used. A unique probe handle was designed and produced by the author. Although it has a similar shape to the handle commercially available for the Aloka probe, it encompasses the important modifications of using disposable needles and having a deeper front end (8 cm). Due to this difference in width the end of the probe handle is kept cranial to the vestibulo-vaginal sphincter and some traction is necessary to remove it from the vagina.

The system was used with disposable 18 G 1½" short bevel needles (Terumo Europe, ref. NN-1838S) and a vacuum of 80-90 mmHg giving an effective suction of 29 ml/min. To test the probe, four cows were aspirated 2 times per week for a period of 5 weeks (total of 40 aspirations). In addition, 21 African buffalo (*Syncerus caffer*) were aspirated. The results are summarised in the table bellow.

	no. of aspirated follicles		no. of recovered oocytes		% recovered
	total	per cow	total	per cow	
cattle	349	8.7	178	4.5	51.0
buffalo	117	5.6	53	2.5	45.3

The system proved to be easy to operate by a single technician working alone. The probe remained in the vagina hands free even if a cow was moving or straining. This was especially an advantage in the buffalo (and would be in other wild species) which were darted with M-99 and were on the ground in a recumbent position. The collector was forced to assume awkward positions and often had to move several times during a TVR session in order to enable the proper positioning of both ovaries. A disadvantage of the deeper front end of the probe handle is that it cannot be used in small bovine heifers or in nulliparous African buffaloes because the vestibulum is too small.

Oocyte recovery rates in cattle were similar to other published data. A recovery rate of 45 % compares, however, favourably with data available from cattle. The ovaries of buffalo appeared to be smaller than the ovaries of cows and had fewer ultrasonographically visible follicles. An average recovery of 2.5 oocytes per aspiration therefore seems to be adequate.

The effect of general anesthesia on renal function in healthy dogs

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Introduction: General anesthesia (GA) has been reported to cause renal dysfunction in dogs. The purpose of this study was to determine the effect of general anesthesia without the concurrent use of intravenous fluids (IVF) on renal function in dogs undergoing ovariohysterectomy.

Materials and methods: Thirty-five healthy dogs over 15 kg were selected. All dogs were premedicated with acetyl promazine (0.05 mg/kg subcutaneously), induced with thiopentone (10-15 mg/kg intravenously), and maintained on 2 % halothane. Only two of the 35 dogs received IVF. Renal function was assessed prior to surgery, 24, and 48 hours after surgery. Renal function and integrity was evaluated using serum urea and creatinine, fractional clearance of sodium (FC_{Na}), urine enzymes (GGT and ALP) and urine analysis.

Results: The duration of the GA ranged from 1 ½ - 5 hours, with an average of 3 hours. There was no change over time in urine specific gravity, serum urea and creatinine, FC_{Na}, and urine enzyme activity, that was indicative of renal dysfunction. The only statistically significant change was a drop in the FC_{Na} between pre-surgery and 24 hours post surgery and between pre-surgery and 48 hours post surgery, possibly as a result of renal retention of water and sodium. Urine enzyme activity expressed as a ratio to creatinine gave minimal variation over time. Seven dogs showed increases in renal tubular epithelial cells on urine sediment examination and concurrent increase in urine enzyme activity, 24 and 48 hours post surgery. However, this was neither a statistically nor a clinically significant finding.

Conclusion: It was concluded that renal function and integrity remained unaffected in this sample of 33 healthy dogs that were not given IVF under GA, as well as the two dogs that were given IVF.

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