UNIVERSITEIT VAN PRETORIA

FACULTY OF VETERINARY SCIENCE
FAKULTEIT VEEARTSENYKUNDE

13th Faculty Day
de Fakulteitsdag

September 27, 1996

PROGRAMME AND SUMMARIES
PROGRAM EN OPSOMMINGS

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

13th FACULTY DAY — 13de FAKULTEITSDAG

27 SEPTEMBER 1996

GEBORG DEUR / SPONSORED BY

PFIZER DIEREGESONDHEID / PFIZER ANIMAL HEALTH

ORGANIZING COMMITTEE / REËLINGSKOMITEE

PROF.R.I. COUBROUGH, (CHAIRMAN), PROF. F. REYERS (CONVENOR), PROF. A.J. BEZUIDENHOUT, PROF. R.A. MEINTJES, PROF. J.S. VAN DEN BERG, PROF. J.J. VAN DER LUGT, DR P.H. TURNER, MR F.J. BEUKES, MR M. HEWITSON, MR F.A. NEL & MISS L. PRINSLOO (SECRETARY)

GEDRUK DEUR V & R DRUKKERY (EDMS) BPK, PRETORIA

INHOUDSOPGAWE CONTENTS

NAVORSINGSPROGRAM (PLAKKATE) / RESEARCH PROGRAMME (POSTERS)

INFORMATION POSTERS / INFORMASIEPLAKKATE

MESSAGE FROM THE DEAN BOODSKAP VAN DIE DEKAAN

Prof. R.I. Coubrough

For some thirteen years, Faculty Day has formed the focal point of our academic year. It has provided a forum for the seasoned and fledgling researcher alike to share the excitement of their research findings or clinical experience with both student and colleague. Faculty Day has contributed significantly to our scientific growth and has become an institution which we must treasure and continue to nurture as an ideal avenue of scientific communication.

The Sir Arnold Theiler Memorial Lecture remains central to the deliberations of the day and this year we are honoured and privileged to have Professor Philip Tobias, eminent scientist and renowned paleo-anthropologist, address us on "Premature Discoveries in Science". A highlight which I have no doubt will place an historic stamp on this special day in our academic calender.

As we enter a new era in our Faculty's illustrious history, we must ensure that Faculty Day continues to grow from strength to strength. As it has become entrenched in our yearly programme, it would be folly to allow it to disappear. In a scientific sense Faculty Day has become sacrosanct and most fitting of a Faculty of stature.

Curriculum Vitae

Prof. Phillip V. Tobias *FRS, FRCP, MBBCh, PhD, DSc (Witwatersrand)*

Professor Phillip V. Tobias is currently Professor Emeritus in the Department of Anatomy and Human Biology at the University of the Witwatersrand, and Director of the Paleo-Anthropology Research Unit at the same university. His illustrious career as teacher and scientist is essentially associated with the Department of Anatomy of the Medical School of the University of the Witwatersrand where, in 1959, he became Professor and Head of the Department, succeeding Professor Raymond Dart. At that time Professor Tobias was the youngest Professor and Head at the University. He also served the Medical School as Dean from 1980 to 1982.

Inspired by Professor Dart, Professor Tobias' research career developed in the fields of physical anthropology and human biology of the living peoples of sub-Saharan Africa, and paleo-anthropology and human evolution based on the study of hominid fossils. He initiated studies at the famous limestone caves at Sterkfontein while his fossil excavations also took place at, amongst others, Makapansgat, Taung and Gladysvale, as well as farther afield in Africa.

Best known for his numerous paleo-anthropological studies, Professor Tobias has documented his findings and philosophy in some nine hundred publications in all categories of the written word (books, scientific articles and popular articles). In recognition of his signal contribution to science, Professor Tobias has been awarded ten Honorary Doctorates, has been visiting Professor at numerous universities across the world and has also been honoured with a great number of medals, prizes and awards. He is a highly sought after speaker and has been invited to present no less then forty-six Eponymous lectures, both locally and internationally. He has also been nominated three times for the Nobel Prize. It is indeed an honour and privilege to have such an eminent scientist present the 1996 Sir Arnold Theiler Memorial Lecture.

07:45 - 08:25 REGISTRASIE EN KOFFIE / REGISTRATION AND COFFEE

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

08:25 - 08:30 VERWELKOMING DEUR SAMEROEPER : REËLINGSKOMITEE / WELCOME BY CONVENOR: ORGANISING COMMITTEE - *PROF. F REYERS*

08:30 - 09:10 FOCUS ON COMPUTER-BASED TESTING / FOKUS OP REKENAAR-GEBASEERDE TOETSING - *MRS IRENE LE ROUX*, UNIT FOR EDUCATIONAL TECHNOLOGY, BUREAU FOR ACADEMIC SUPPORT SERVICES, UNIVERSITY OF PRETORIA

09:10 - 09:15 OPENINGSREDE EN VOORSTELLING VAN PROF. P.V. TOBIAS / OPENING AND INTRODUCTION OF PROF. P.V. TOBIAS
PROF. R.I. COUBROUGH, DEKAAN

09:15 - 10:10 SIR ARNOLD THEILER MEMORIAL LECTURE / SIR ARNOLD THEILER-GEDENKLESING : *"PREMATURE DISCOVERIES IN SCIENCE" - PROF. PHILLIP V. TOBIAS*

10:15 - 10:25 TOEKENNING AAN "DOSENT VAN DIE JAAR" / "LECTURER OF THE YEAR" AWARD OPVSK VERTEENWOORDIGER / OPVSC REPRESENTATIVE

10:25 - 10:40 UNVEILING OF BUSTS OF FORMER DEANS / ONTHULLING VAN BORSBEELDE VAN VOORMALIGE DEKANE - *MNR DAAN COETZER*

10:40 - 11:00 TEE EN PLAKKAATBESIGTIGING / TEA AND VIEWING OF POSTERS

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SESSION CHAIRMAN: PROF. G.F. BATH : SESSIEVOORSITTER

1. The effect of management schemes on helminth levels in donkeys used as traction animals in Hammanskraal - *D. Wells, R.C. Krecek & J.C. Lourens*

2. Rabies trends in South Africa: 1985-1994 - *M. More O'Ferrall-Berndt & B. Gummow*

3. A molecular epidemiological investigation into the role of *Salmonella* serovars in perinatal mortalities in a group of captive cheetah - *M. van Vuuren, E.H. Venter & J. Carstens*

4. The immobilisation stress response of the African wild dog, *Lycaon pictus* - *M.S. de Villiers, D.G.A. Meltzer, M.G.L. Mills, A.S. van Jaarsveld & P.R.K. Richardson*

12:00 - 13:00 MIDDAGETE (VIR GEREGISTREERDE DEELNEMERS) / LUNCH (FOR REGISTERED PARTICIPANTS)

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SESSION CHAIRMAN : PROF. A.J. GUTHRIE : SESSIEVOORSITTER

FRD Funding Information - *Prof. R.I. Coubrough*

5. The isolation and identification of microorganisms from faeces of dogs with gastroenteritis - *E.H. Venter, J.J. Gouws, J.H. Carstens, G.H. Goosen, E. Styliandes & P.G. Howell*

6. Immunophenotypic classification of canine malignant lymphoma on formalin fixed paraffin wax embedded tissue using CD3 and CD79a cell markers - *R.J. Milner, J. Pearson, J.W. Nesbit & P. Close*

7. Met-haemoglobinuria in canine babesiosis - *R.G. Lobetti & F. Reyers*

8. Occurrence of *in utero Babesia equi* carrier infections in the offspring of known carrier mares - *B.D. Lewis, B.L. Penzhorn, M.T.E.P. Allsopp, D.H. Volkmann, & A.J. Guthrie*

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SESSIEVOORSITTER : PROF. A.J. BEZUIDENHOUT : SESSION CHAIRMAN
PLAKKAATBESIGTIGING EN AANBIEDING / POSTER VIEWING AND PRESENTATION

14:45 - 15:15 REFRESHMENTS / VERVERSINGS

15:15 - 16:15 NAVORSINGSPROGRAM : SESSIE III / RESEARCH PROGRAMME : SESSION III
SESSIEVOORSITTER : PROF. B.L. PENZHORN : SESSION CHAIRMAN

9. Association of noctuid eye-frequenting moths with ophthalmia in cattle - *J.J. Gouws, J.A.W. Coetzer & P.G. Howell*

10. The detection and inactivation of certain pathogens in cows' milk that can affect human health - *J.G.K. Kangumba, E.H. Venter & J.A.W. Coetzer*

11. NAD independence : A possible mechanism of immuno-evasion by *Haemophilus paragallinarum*? - *R.R. Bragg, J.M. Greyling, T.C. Beer & J.A. Verschoor*

12. *Escherichia coli* heat-stable enterotoxin alters the uptake of solutes in the rabbit jejunum *in vitro* - *D.B. Petty, J.G. van der Walt & H.E. Engelbrecht*

16:15 - 16:30 DEAN'S AWARDS : BEST PAPER, BEST POSTER AND PHOTOGRAPHIC EXHIBITION AWARDS / DEKAANSTOEKENNINGS : BESTE REFERAAT, BESTE PLAKKAAT EN FOTOGRAFIESE UITSTALLING TOEKENNINGS

16:30 - 19:30 AFSLUITING EN SKEMERPARTYTJIE / CONCLUSION AND COCKTAILS

The effect of management schemes on helminth levels in donkeys used as traction animals in Hammanskraal*

D. Wells, R.C. Krecek & J.C. Lourens

Department of Veterinary Tropical Diseases

In Southern Africa, where 300 000 working equids provide an important alternative to mechanisation in resource-poor communities, very little is known about the helminth status of these donkeys, or about the impact of helminths on work output. The aim of this study was to investigate the helminth status of working donkeys under different management systems and the effects of these worms on body mass and condition of the donkeys.

Donkey owners in three different areas (one rural and two semi-rural) of Hammanskraal were visited and interviewed to assess their management systems. The identity, height, heart girth, length and condition score of each donkey participating in the study was recorded at the same time that a faecal sample was collected from the donkey. This was repeated once a month over one year. Faecal samples were analysed of nematode eggs, trematode eggs and cultured to identify nematode species.

Final comparisons between management system subgroups for age, mass, condition score, faecal egg count, species composition of larval cultures and work done by die donkeys were performed. Two management systems were identified, and differences in body mass and condition seore of donkeys under the two systems were found. Helminth species composition and faecal egg count numbers also differed between the three areas.

Since the results showed differences in the number and species of helminths in donkeys kept under different management systems, it may be possible to suggest a management system which would be appropriate for the control of helminth parasites in donkeys.

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* *Research Project No. 36.5.175. Approved by the Faculty Ethics and Research Committees.*

Rabies trends in South Africa: 1985-1994*

M. More O'Ferrall-Berndt & B. Gummow

Department of Veterinary Public Health

Animal and human rabies trends in South Africa for the period 1985 to 1994 were determined. The records of the National Institute for Virology, the Onderstepoort Veterinary Research Institute and the Allerton Regional Laboratory were analysed, using standard epidemiological techniques and the CDC programmes Epi Info and Epi Map.

There was an increase in the total number of positive cases recorded, both in animals and humans. This was predominantly due to a rabies epidemic in KwaZulu-Natal where 42 % of all animal cases occurred. Companion animals made up 51 %, production animals 20 % and wildlife 29 % of reported animal cases of rabies. From a public health point of view, a disturbing trend developed in that the incidence in domestic animals increased whereas the actual amount of wild animal cases remained constant over the period under review.

Mortality rates were highest in children under twenty years of age (0,65 per 100 000) and in those over sixty years (0,55 per 100 000). The majority of cases (87,2 %) were attributed to dog bites. During the period under review there was also an increase in the number of people having been bitten by a confirmed positive rabid animal (0,15 to 0,41 per 100 000). Seasonal trends showed peaks in the winter months for animals and peaks in the summer months for human victims.

Even though human rabies cases only account for 3 % of all reported cases, it is on the increase, and therefore rabies in animals needs to be eradicated, or at least controlled to prevent its transmission to humans. The current trends suggest that existing control measures for canine rabies in South Africa are not adequate and need to be critically reviewed.

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* *Research Project No. 36.5.183. Approved by the Faculty Ethics and Research Committees.*

A molecular epidemiological investigation into the role of *Salmonella* serovars in perinatal mortalities in a group of captive cheetah*

M. van Vuuren, E.H. Venter & J. Carstens

Department of Veterinary Tropical Diseases

Survival rate of new-born free-living cheetah is reported to be as low as 30 %. In breeding establishments birth rates are similarly low, but detailed causes of abortion and stillbirth have not been reported.

A large number of strains of *Salmonella* were isolated from a wide variety of specimens obtained from a cheetah breeding establishment. It is postulated that the serovars isolated were involved in perinatal mortalities experienced in a group of captive cheetah, and that they were introduced into the host animals by means of contaminated meat. This study examined the possible role of *Salmonella* in abortion and stillbirth in the group of captive cheetah.

All *Salmonella* isolates obtained from the diets and faeces of the cheetahs as well as aborted foetuses and the environment, were serotyped. Serovars were then subjected to random amplified polymorphic DNA (RAPD) fingerprinting to compare DNA amplification fingerprints of the genomic DNA.

Preliminary results of the comparison of the polymerase chain reaction (PCR) amplified products showed that random amplified polymorphic DNA fingerprinting can distinguish between strains of the same bacterial species.

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* *Research Project No. 36.5.222. Approved by the Faculty Ethics and Research Committees.*

The immobilisation stress response of the African wild dog, *Lycaon pictus*

M.S. de Villiers, D.G.A. Meltzer[1], M.G.L. Mills[2], A.S. van Jaarsveld[2] & P.R.K. Richardson[2]

National Parks Board, Skukuza, [1]Price Forbes Chair in Wildlife Diseases,
[2]Department of Zoology & Entomology

The African wild dog, *Lycaon pictus*, is an endangered species with a maximum of 5000 individuals left in the wild. Captive breeding institutions have the potential to make a valuable contribution to wild dog conservation by maintaining a population of animals which is physically, genetically and behaviourally healthy. In order to determine basic approaches towards the captive breeding and reintroduction of the species, an investigation of captive and free-ranging wild dogs was undertaken. We report on the assessment of chronic stress exposure in wild dogs through the measurement of the plasma cortisol response of individuals to the controlled acute stress of chemical immobilisation.

Captive wild dogs were maintained under a variety of conditions, ranging from small groups held in small, adjacent enclosures (KENNEL animals), to a pack of animals held in a three hectare camp. Free-ranging wild dogs were from five packs in the Kruger National Park. Stereotypic behaviour of captive animals was recorded using focal animal sampling. Social dynamics of the captive pack were recorded using *ad lib* sampling. Dominance hierarchies were constructed according to the direction of agonistic and affiliative interactions.

Animals were darted with a combination of fentanyl and xylazine. An initial blood sample was collected as soon as possible after darting, and serial samples were drawn at ten minute intervals for 70 minutes. ACTH was then administered to members of the captive pack and further samples were collected at 20 minute intervals for the next two hours. Plasma was harvested and stored at - 20°C until assayed with a validated commercial I^{235} radio-immunoassay kit.

Initial and peak plasma cortisol concentrations were highest in KENNEL animals, which also had cortisol profiles indicative of regular stress exposure. These animals paced excessively and had low body weights. It was hypothesised that animals held in adjacent enclosures experience extreme stress due to their inability to resolve social relationships, and that this may affect their health, behaviour and reproductive potential.

There was no clear relationship between cortisol measures and rank. Instead, cortisol concentration was negatively correlated with age, i.e. younger animals had higher concentrations. For males, this difference was especially apparent during the latter half of the study period when young males occupied high-ranking positions in the hierarchy. While the cause of variation in cortisol response remains speculative, it may be due to individual differences in social skilfullness so that older, more experienced individuals find dominance interaction more predictable and hence less stressful than younger individuals.

Mortalities of wild dogs in the Serengeti ecosystem gave rise to the hypothesis that the stress of handling (immobilisation and rabies vaccination) of wild dogs by researchers resulted in immunosuppression, the reactivation of latent viruses and eventually, death. Cortisol profiles of free-ranging and captive wild dogs indicated that handling did not cause the chronic stress required by this hypothesis. The mortalities in Serengeti were more likely due to an outbreak of a disease which vaccination was unable to prevent.

The isolation and identification of microorganisms from faeces of dogs with gastroenteritis*

E.H. Venter, J.J. Gouws, J.H. Carstens, G.H. Goosen, E. Styliandes & P.G. Howell

Department of Veterinary Tropical Diseases

Gastroenteritis in dogs is of a multi component nature. Parvovirus, however, in spite of vaccination, remains an important cause of this syndrome and is often implicated in fatal disease in young puppies. An investigation into the prevalence of the virus as well as the presence of specific bacteria in cases of dogs with gastro-enteritis was carried out.

Samples from dogs with gastroenteritis were collected from the Veterinary Academic Hospital. Faeces samples (n=125) as well as blood in heparin and for serum, were collected. Faeces samples were suspended in sterile saline and distributed to the Bacteriology, Virology and Serology sections of the Department and negatively stained preparations were also examined by electron microscopy. The aerobic isolation of bacteria as well as the selective isolation of *Salmonella*, *Clostridia* and *Campylobacter* were carried out. The haemagglutination test to detect parvovirus was used on faeces samples while the haemagglutination inhibition test was used to detect antibody in serum samples. For viral isolation NLFK cells (feline kidney) were inoculated using heparin blood and the presence of parvovirus in cell cultures was confirmed by a direct fluorescence test using a commercially available conjugate.

Clostridium was present in 27 % of samples, while no *Campylobacter* was isolated from the faeces. Other aerobic bacteria included *Salmonella* (4 %), *Klebsiella* and smooth *E. coli*. Using an electron microscope, 78 % of faeces samples were positive for parvovirus, while 54 % of faeces samples were positive using the haemagglutination test and 92 % of serum samples were positive for specific parvovirus antibody in the inhibition test. There was a good correlation between viral isolation and results obtained from the electron microscopic examination.

Future studies will involve the extraction of DNA from the isolated parvo viruses and their comparison to the DNA of viruses in the existing vaccine.

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* *Research Project No. 36.5.112. Approved by the Faculty Ethics and Research Committees.*

Immunophenotypic classification of canine malignant lymphoma on formalin fixed paraffin wax embedded tissue using CD3 and CD79a cell markers

*R.J. Milner, **J. Pearson**[1], J.W. Nesbit[1] & P. Close[2]*

Department of Medicine,[1]Department of Pathology, [2]Department of Anatomical Pathology, University of Cape Town

Canine malignant lymphoma (CML) is a fairly common lymphoid neoplasm. Identification of the immunophenotype is of prognostic importance; T cell lymphomas have a worse prognosis than B cell lymphomas. Until recently, identification of T or B cell lymphomas was undertaken utilising flow cytometry or fluorescent immunocytochemistry on frozen sections. Whilst being used in the research field, these methods are impractical for routine diagnostic histopathology because of the type of specimens (formalin fixed tissue) that are normally received by pathologists. Commercially available CD3 antibody has been used successfully to identify T cells in formalin fixed paraffin wax embedded tissue sections. B cells are identified with a recently, commercially available pan B cell marker, CD79a; suitable for diagnostic application using formalin fixed paraffin embedded material. Antibody markers to CD3 and CD79a show cross-reactivity across species lines for T and B cells, respectively.

These markers were used successfully to distinguish histopathologically between T and B cell lymphomas. Out of the five cases that were examined, three reacted positively with CD3 (negative with CD79a) and two reacted positively with CD79a (negative with CD3). The positively of the latter marker showed a strong correlation with the results yielded by more conventional B cell markers such as anti-IgG, anti-IgM, and IgG.

Apart from the obvious advantages for the veterinary diagnostic histopathologist, the advantage of accurate phenotyping will also extend to veterinary research.

Met-Haemoglobinuria in canine babesiosis*

R. G. Lobetti & F. Reyers

Department of Medicine

It has been shown in dogs that haemoglobinuria *per se* does not cause renal damage but that met-haemoglobinuria, especially in the presence of aciduria is nephrotoxic. The purpose of this investigation was to compare whole blood and urine met-haemoglobin fractions, as well as whole blood haemoglobin levels and urine pH from dogs with naturally occurring *B. canis* infection, showing visible haemoglobinuria, with that of clinically healthy dogs.

Anaerobic urine and heparinised blood samples were collected from control dogs (n=5) as well as dogs with naturally occurring *B. canis* infection, showing visible haemoglobinuria (n=6). The control group comprised five clinically healthy dogs. The six dogs with *B. canis* infection were all presented to the Outpatients Clinic of the Onderstepoort Veterinary Academic Hospital of the Faculty of Veterinary Science. For inclusion in the trial, these dogs had to have confirmed *B. canis* infection, diagnosed on a stained, thin capillary blood smear; be in-saline agglutination negative; and had to have visible haemoglobinuria. The urine colour varied from red-brown to purple-black. Urine and blood samples were collected prior to the administration of any therapeutic agents and both samples were assayed within 30 minutes after collection. A haemoximeter was used to determine the met-haemoglobin fractions in the blood and urine. A blood gas analyser was used to determine the urine pH. The instruments were calibrated using standard calibration procedures and standard solutions. The data from the control and *B. canis* infection groups were compared using analysis of variance. Significance was set at $p < 0,05$.

The group means of the urine haemoglobin, blood haemoglobin, urine met-haemoglobin and urine pH from the dogs with *B. canis* infection differed significantly from that of the control dogs. The urine pH, and blood haemoglobin levels were significantly lower in infected than in control animals. The means of the blood met-haemoglobin did not differ significantly between the two groups. The urine haemoglobin concentration ranged from 1-4 $g.\ell^{-1}$ which was not related to the blood haemoglobin concentration nor the haematocrit. Only two cases showed detectable blood met-haemoglobin fractions, although the amounts were negligible (0,9 and 1,3 %). Urine met-haemoglobin fractions ranged from 28-59 %. Although the urine pH in the *B. canis* infected group was lower than that of the control group, it was not significantly different.

This study revealed that dogs with *B. canis* infection showing haemoglobinuria have significant met-haemoglobinuria. This finding may partly explain the occurrence of renal disease in canine babesiosis.

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* *Research Project No. 36.5.194. Approved by the Faculty Ethics and Research Committees.*

Occurrence of *in utero Babesia equi* carrier infections in the offspring of known carrier mares*

B.D. Lewis, B.L. Penzhorn, M.T.E.P. Allsopp[1], D.H. Volkmann[2], & A.J. Guthrie[3]

Department of Veterinary Tropical Diseases, [1]Molecular Biology Section, Onderstepoort Veterinary Institute, [2]Department of Theriogenology, [3]Equine Research Centre

Very little is known about how and why *Babesia equi* related abortions occur, or about the host-parasite interaction *in utero*. Classical theories suggest that once infected, the foetus is destined to be aborted. If one looks at other protozoan and rickettsial parasites it becomes obvious that this need not necessarily be the case. For example, bovines infected with *Anaplasma marginale* may give birth to clinically healthy calves which are parasite carriers. The trial reported on here aims to investigate whether a *B. equi* carrier state, similar to the *A. marginale* carrier state can be set up *in utero*, and to investigate whether the natural intake of colostrum has any effect on this infection.

Six foetuses in different stages of gestation were mechanically aborted from known *B. equi* carrier mares. Polymerase chain reactions were carried out on DNA extracted from the foetal spleens. Amplification products were then blotted and probed with a radio labelled *B. equi*-specific probe. A similar procedure was adopted with DNA extracted from blood collected from seven newborn foals of less than 24 hours old. All foetal spleen and newborn foal DNA samples were positive for *B. equi*, indicating the presence of an *in utero B. equi* carrier infection. A *B. equi* carrier mare was placed in a tick-free isolation unit prior to parturition. Blood samples were collected from the foal prior to colostral intake, and then daily for 21 days thereafter. The samples were analysed for the presence of *B. equi* using the DNA probe technique. Parasite DNA was found to persist in the foal's blood for the entire 21 days of the trial.

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* *Research Projects No. 36.5.168 and No. 36.5.197. Approved by the Faculty Ethics and Research Committees.*

Association of noctuid eye-frequenting moths with ophthalmia in cattle

J.J. Gouws, J.A.W. Coetzer & P.G. Howell

Department of Veterinary Tropical Diseases

A project was designed to determine the possible association of noctuid eye-frequenting moths with ophthalmia in cattle and to compare the microflora of clinically healthy and affected bovine eyes.

Swabs were collected once a month over a one year period from 414 healthy and 64 affected bovine eyes from a farm near Brits and cultured for bacteria and mycoplasmas. Antimicrobial sensitivity tests were done on five isolates of each of the four most common bacteria isolated from clinically affected eyes.

On three occasions during the study period eye-frequenting moths were collected, identified and cultured for bacteria and mycoplasmas. Scanning electron microscopy was done on the proboscis of both species (*Arcyophora longivalvis* and *A. patricula*) of moths collected.

Bacteria were isolated from 48,6 % healthy eyes and 87,5 % affected eyes. No bacteria were isolated from the remaining eyes. Eleven genera of bacteria were isolated from healthy eyes and eight genera from affected eyes. The majority of isolates were classified in the genera *Moraxella*, *Neisseria* and *Staphylococcus* : *Moraxella bovis* was isolated most commonly. Mycoplasmas were isolated from 50,7 % healthy eyes and 42,2 % affected eyes. Ureaplasmas were not isolated from any of the eyes. Antimicrobial sensitivity tests showed that the bacterial isolates were most susceptible to gentamycin, chloramphenicol, neomycin, amikacin, kanamycin, cephalothin and erythromycin.

Twelve different genera of bacteria were isolated from 21 eye-frequenting moths. Nocardia, Corynebacterium, Staphylococcus, Moraxella and *Mycoplasma* spp. were most frequently found. *M. bovis* was isolated from one moth.

Scanning electron microscopical studies of the proboscis of the moths showed it to contain various sensillae and short triangular denticles which include galeal linkage plates, sensillae styloconica, triangular cuticular processes, sensillae trichodea and sensillae basiconica.

Moraxella bovis was isolated most frequently of all the bacteria found in healthy and affected eyes. It was also found to have the highest prevalence in affected eyes indicating that it can be pathogenic when conditions are favourable in the eye.

The proboscis of the eye-frequenting moth contains sensillae and denticles which could possibly cause injury to the mucous membranes of the eye and predispose to ophthalmia in cattle.

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* *Research Project No. 36.5.38. Approved by the Faculty Ethics and Research Committees.*

The detection and inactivation of certain pathogens in cows' milk that can affect human health*

J.G.K. Kangumba, E.H. Venter[1] & J.A W. Coetzer[1]

Milk Hygiene Section, Onderstepoort Veterinary Institute, [1]Department Veterinary Tropical Diseases

Milk-borne zoonotic risk factors are of great concern, particularly with regard to resource-poor communities where the installation of, or access to, cooling facility is almost impossible mainly due to socio-economic conditions. An investigation into the feasibility and efficiency of alternatives to pasteurization and refrigeration of milk was carried out.

Fifty milk samples from ten cows were collected in five groups of ten samples each. Each cow was represented in every group by one sample. Each sample within a group was divided into four samples (A,B,C,D) and infected with a known bacterial concentration from one of five target pathogens: *S. aureus, E. coli, B. abortus, M. bovis* and *C. burnetii*. Samples A from each group were subjected to the activation of the lactoperoxidase-system (LP) for six hours at 30°C; samples B to a souring procedure for 24 hours at 30°C and samples C to heat treatment as a negative control for 30 minutes at 65°C. Samples D were not treated (positive control). At the end of the treatment the cell concentration of the pathogens in milk was determined. The survival/kill rate of pathogens was calculated using the BMDP 3D statistical package.

The inhibitory effect of the LP-system activation and the souring of milk was very significant for *S. aureus* and *E. coli* organisms, in which a growth retardation of at least two log values in the cell concentration was obtained after six hours of treatment as compared to the growth pattern of the organisms in the positive control. No growth was obtained from the heat-treated negative control.

This study generated baseline data which might lead to field trials using the two above mentioned treatments. This could lead to the use of alternatives to refrigeration in areas where cooling facilities are not accessible.

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* *Research Project No. 36.5.177. Approved by the Faculty Ethics and Research Committees.*

NAD independence : A possible mechanism of immuno-evasion by *Haemophilus paragallinarum*? *

R.R. Bragg, J.M. Greyling, T.C. Beer & J.A. Verschoor[1]

Department of Poultry Diseases, [1]Department of Biochemistry

Nicotene-adenine-dinucleotide (NAD) independent *H. paragallinarum* were first isolated from poultry in South Africa in 1990. Since then, they have been isolated with increasing frequency.

These NAD independent isolates were tested with a panel of mAbs in ELISA, and by haemagglutination (HA) and haemagglutination inhibition (HI) tests, using rabbit polyclonal antisera.

Reaction patterns of the mAbs in ELISA were very similar to those obtained with the NAD dependent field isolates. One interesting exception was eight isolates which reacted with the V1 mAb. It was also established, from the HI tests, that a large percentage of the NAD independent isolates were serovar A-1, with some serovar C-3 and very few serovar C-2. From these results, it is possible to speculate that the NAD independent *H. paragallinarum* isolates have developed a mechanism to evade the immune response of the host.

None of the South African field isolates of NAD dependent *H. paragallinarum* reacted with the V1 MAb. Only the reference strain 0083, and two Australian field isolates were found to react with the V1 MAb. All of the infectious coryza vaccines used in South Africa contain serogroup A strains and in many cases, strain 0083 is used. It is therefore postulated that the use of vaccines has eradicated isolates expressing the antigen detected by the V1 MAb. This is substantiated by the fact that NAD dependent V1 expressors were found in Australia, where no vaccines containing 0083 have ever been used.

Further evidence of possible immune evasion can be seen when the incidence of the different serovars among the NAD dependent and the NAD independent isolates are examined. Serovar A-1 has all but disappeared in the NAD dependent isolates, possibly as a result of the long term use of vaccines containing serovar A-1, while in the NAD independent isolates, serovar A-1 predominates. All of the NAD independent isolates have been made from vaccinated chickens, which should be protected against serovar A-1 challenge.

It is further postulated that the ability of the isolates to grow without NAD has opened new niches in the sinuses of the chickens, possibly in areas devoid of blood and therefore circulating antibodies. A new strategy is therefore possibly needed for the control of the NAD independent isolates.

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* *Research Project No. 36.5.221. Approved by the Faculty Ethics and Research Committees.*

Escherichia coli heat-stable enterotoxin alters the uptake of solutes in the rabbit jejunum *in vitro*[*]

D.B. Petty, J.G. van der Walt & H.E. Engelbrecht

Department of Physiology

Introduction *E.coli* heat-stable enterotoxin (STa), mimicking the actions of the hormones guanylin and uroguanylin binds to GC-C receptors in the gut. This leads to the activation of cGMP which is associated with chloride and water secretion as well as depressed sodium uptake. Brandsch *et al* working on Caco-2 cells demonstrated an inhibition of taurine but not glucose uptake in the presence of *E. coli* heat-stable enterotoxin. Since it has been suggested that there is enhanced ability to absorb solute as an adaptation to maximize water absorption in desert rodents, the investigators hypothesized that guanylin, a hormone known to affect water and electrolyte balance in the intestine could affect solute uptake in the small intestine.

Each rabbit (n=7) was anaesthetized with pentobarbitone sodium (Sagatal), a section of the jejunum was exteriorised and perfused with ice-cold Ringer's solution prior to its removal. Upon removal it was cut into a number of pieces and everted onto glass rods and kept in ice-cold oxygenated Ringer's solution until the commencement of the experiment. Thereupon it was placed in varying concentrations (1-20mmol) of glucose or lysine, with or without the STa toxin.

The results show that the uptake of glucose was increased by the presence of STa toxin while the uptake of lysine was largely unaffected by the presence of the STa toxin over the range of concentrations tested.

The results suggest that STa toxin and therefore guanylin and uroguanylin could affect the uptake of solutes in the gut.

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The effect of gassing, preincubation time and incubation time in the *in vitro* everted gut method of Karasov and Diamond*

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There are a number of techniques for studying uptake of solutes in the isolated small intestine. The *in vitro* technique of Karasov and Diamond is a modification of the everted sleeve technique and differs in that it places the everted gut over a grooved glass rod which is then placed in a jacketed water bath kept at 37°C and stirred by a magnetic stirrer at a rate of 1200 rpm. This enables the measurement of uptake across the mucosal surface without the complicating factor of the serosal surface. The disadvantage of any *in vitro* method is that the tissue, having been removed from the body, is susceptible to hypoxia.

Each rabbit was anaesthetized with pentobarbitone sodium (Sagatal), a section of the jejunum was exteriorised and perfused with ice-cold Ringer's solution prior to its removal. Upon removal it was cut into a number of pieces and everted onto glass rods and kept in ice-cold oxygenated Ringer's solution (preincubation) until the commencement of the experiment Thereupon each everted sleeve was placed in varying concentrations (1 or 50 mmol) of labelled glucose or lysine for four minutes (incubation) and the uptake of solute per mg of tissue per minute was determined.

In the first experiment the length of time the tissue was kept in iced Ringer's solution prior to the commencement of the experiment (preincubation time) was varied between 40 and 220 minutes (n=8). In the second experiment, the length of time that the tissue was kept in the test solution (incubation time) was varied between two and ten minutes (n=5). In the third experiment the effect of changing the rate of gassing or alternatively using a magnetic stirrer during the incubation period was measured (n=6).

The results show that the uptake rates were constant in tissue kept in oxygenated Ringers solution for a period of up to 120 minutes after the tissue has been removed from the animal. Uptake rates progressively decreased with increasing incubation time. The use of either a magnetic stirrer or the rate of gassing had a pronounced effect on uptake rates, with the best result being obtained when using a magnetic stirrer or vigorous gassing and the worst results being obtained when the solutions were not gassed at all.

It is concluded that the everted sleeve preparation is viable for at least 120 minutes after it has been removed from the animal and that mixing and incubation time have a major effect on uptake rate in this isolated gut preparation.

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Prevalence of coccidia affecting small ruminants especially indigenous goats mainly grazing on communal land*

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Small ruminant stock play a major role in protein production in rural communities. The diseases and parasites of these animals have not been investigated in detail. The aim of this project was to determine the prevalence of *Eimeria* spp. in small ruminant stock in three selected herds located in the northern part of South Africa, as well as determining which *Eimeria* spp. occur in the afore-mentioned animals.

Three locations were chosen, each having a herd containing indigenous goats. These were Medunsa, Nebo and Hammanskraal. Faecal samples were collected randomly from different age groups within these herds on a monthly basis. The animals included indigenous goats, Saanen goats, Saanen-indigenous crossed goats and indigenous sheep. Oocysts per gram of faeces was calculated for each sample, using the MacMaster technique and species identification was done microscopically following sporulation of oocysts in 2,5 % potassium dichromate.

Eimerian oocysts were found in 95,26 % of 1372 faecal samples. Preliminary results indicate an increase in oocyst excretion in the adult goats over the period November to March in all three areas coinciding with the rainfall and warm weather. The kids excreted far higher numbers of oocysts than the adults, the highest number for an individual animal being 930 400 OPG. The most prevalent Eimerian species was *E. arloingi* followed by *E. hirci* and *E. christenseni* respectively.

Diarrhoea was noticed to play a major role in the disease picture seen in young kids in kraals, causing both unthriftiness and death. Although coccidiosis may be implicated, severe worm infections had also been seen in many of these cases.

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* *Research Project No. 36.5.198. Approved by the Faculty Ethics and Research Committees.*

The development and use of a group specific polymerase chain reaction for bluetongue virus*

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Bluetongue virus the prototype virus of the Orbivirus genus consists of a segmented double stranded RNA genome. Twenty four serotypes have been isolated worldwide of which at least 21 may be found in South Africa. The polymerase chain reaction (PCR) has been standardised for use as a group specific diagnostic test for the identification of the virus.

The gene coding for the non-structural protein NS1 has been found to be the most conserved gene within all the serotypes and was used to select primers for the PCR. Primers were selected from BTV serotype 4 (BTV4) and this serotype was also used to standardise the protocol.

BHK-cells were inoculated with BTV4 and after the monolayer showed advanced cytopathology, the cells were harvested and the virus quantified by plaque assay. A total RNA extraction of the harvested cells was carried out according to an acid guanidinium thiocynate-phenol-chloroform extraction method of Chomczynski and Sacchi (1987). The total RNA was used as template material for the PCR.

The optimum annealing temperature for the primers was established. The concentration of both the primers and the template as well as the number of cycles necessary for the PCR were parameters used to standardise the test.

The best results were obtained at an annealing temperature of 52°C with a template concentration of 140 plaque forming units per μl for 35 cycles.

The test could be used as a diagnostic test for detecting BTV in field samples e.g. BTV infected blood and Culicoides midges and will be able to detect all SA serotypes. The evaluation of the primers and the standardised reaction conditions to detect the five American BTV serotypes will be done in the near future.

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Aerobic bacterial population of chicken carcases at selected critical control points in a processing plant

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The poultry industry plays an increasingly important part in the animal food market. The process of converting live chicken into poultry products is susceptible to contamination of carcases by micro-organisms from the environment, equipment, human handling and through product cross-contamination (soiling).

A pilot study was conducted on three broiler carcases selected from critical control points in a South African Grade B poultry abattoir to determine base line data on the microbiological status. The daily throughput varies up to 3 000 birds and the processing is labour intensive. The critical sites chosen were after defeathering, after evisceration and after chilling. The locations of sampling on the chicken carcases were as follows: neck skin, meat from both sides ventral to the wings, skin from both sides ventral to the wings, meat caudal to breastbone, skin caudal to breastbone, skin cranio-dorsal to pygostyle, skin from back between the wings and skin ± 2 cm cranial to the centre of the breast.

Total bacterial counts done on the samples from the carcases show that the greatest bacterial counts were obtained from the samples taken from the neck skin of the carcases and incubated at 37°C. The organisms were identified and the dominant Gram negative organisms were *E. coli, Proteus vulgaris, Acinetobacter baumanii* and *Citrobacter intermedius*. The dominant Gram positive organisms were *Staphylococcus saprophyticus, Staphylococcus aureus* and *Enterococcus cecorum*.

Findings include

- Bacterial contamination levels of carcase skins were high.
- Spoilage bacteria *Pseudomonas* and *Acinetobacter* will lower shelf life.
- Pathogenic genera *Escherichia* and *Staphylococcus* could pose a health risk to the consumer.

Parentage puzzle

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An American Saddler exported to the USA had to be parentage tested to verify that this was the correct horse.

As the dam was dead, several half-sibs and their sires were blood typed to compile the blood picture of the dam. Acetate dextrose tubes (7ml) were used for 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 alleged sire was then excluded and several other possibilities were investigated. A young stallion was present on the stud-farm with the alleged sire. A stallion was also present on the previous farm with the mare. Both had since died without being blood typed. Family of the latter were, however, available and the blood picture could be compiled on which he could not be excluded as a possible sire.

As a result of blood typing exclusion could be given in this case.

Outbreak of "twisted leg syndrome" in ostrich chicks

A. Shakespeare

Department of Medicine

An attempt to try and determine the cause of an outbreak of "tibio-tarsal rotation" syndrome in a group of Ostrich chicks is reported.

Eight ostrich chicks (8 to 12 weeks old) were presented to the department with a variety of clinical signs. These included deformed rib cages, enlarged joints, slipped tendons, rotated legs and pathological fractures of the legs. Various techniques including xero-radiography, blood mineral determination, bone mineral determinations, dietary analysis and liver mineral determinations as well as response to treatment were attempted.

Serum values from the eight birds resulted in normal calcium but significantly depressed serum inorganic phosphate levels in all the samples. Despite treatment with intra-venous phosphorus and bone meal *per os* the serum inorganic phosphate levels remained low. Liver determinations from two birds revealed significantly decreased calcium, copper, manganese and zinc levels. Dietary analysis indicated a high calcium content with a calcium: phosphate ratio of 2:1. Bone calcium and phosphorus determinations from three affected and two normal birds were unsatisfactory (histopathology of joints is still pending).

The problem appears to be dietary related. The high calcium and protein levels potentiate leg problems which are accentuated by deficiencies in manganese, zinc and possibly copper. Limited exercise in conjunction with the hot diet (high protein levels) has been proposed as a major cause of the above syndrome. Prevention of this problem in future chick raising ventures is important and will include:

a) increased exercise
b) decreasing $Ca:PO_4$ ratio via withdrawal of shell grit and/or the addition of sodium mono-phosphate @ 8 kg/ton of feed
c) addition of copper @ 200 g/ton feed
d) addition of Zinc and Manganese @ 140 g/ton feed
e) decreased boredom (i.e. add stones, sun, foster parents, etc.)

A suggested lateral surgical approach to the guttural pouch of the horse based on anatomical findings*

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Veterinary students BVSc III, [1]Department of Anatomy

There are four existing approaches to the guttural pouches in the horse. Each approach requires skilful surgery as they all have potentially sensitive structures to pass. There is, however, an area on the lateral aspect of the pouch where no major blood vessels or nerves are found. The aim of this project therefore is to utilise this site surgically in a lateral approach to the pouch as it will cross muscle and bone, avoiding "critical" structures.

Three detailed dissections have been performed on formalin-fixed specimens. These were formative dissections and were used for reference. The current work is being conducted on unfixed horse heads for statistical analysis and to verify that the site has the same anatomical location in all horses, regardless of age, sex, and breed. It is also aimed to determine an approach that will enable a practitioner to utilise this site for quick and easy access for any procedure.

The site is determined by extrapolating a line along the occlusion surface of the teeth in a caudal direction and a perpendicular line from the tempero-mandibular joint. The caudal dorsal border of the site is located at the intersection of these two lines. Using a checklist, various parameters (morphometric data, signalment, nerves, blood vessels and miscellaneous structures) were recorded. These will determine the safety margins and confirm the approach to this site.

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Stress-related effects in blood variables in shechita-slaughtered cattle

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In order to reduce the stress involved with slaughter, animals are routinely rendered unconscious prior to slaughter. Captive bolt stunning is the principle method of pre-slaughter stunning of cattle. In South Africa, all animals slaughtered at an abattoir must be stunned prior to exsanguination, with the exception of shechita-slaughtered animals (Act no. 87 of 1967). According to Jewish law, an animal must be healthy and uninjured prior to slaughter; therefore shechita slaughter cannot employ captive bolt stunning as this is classed as a form of injury. Nevertheless, at the Johannesburg abattoir even shechita-slaughtered animals are stunned, although only after exsanguination. The aim of this study is to determine the effectiveness of the captive bolt stun in reducing the stress associated with slaughter.

Two groups of cattle were slaughtered according to the shechita method. The cattle in group 1 (n=22) were stunned within ten seconds after exsanguination, whereas those in group 2 (n=18) were not stunned. Blood samples were collected as the throat was cut (T=0s), and then between 60 seconds and 120 seconds later (T=60s). Plasma variables associated with the stress response (haematocrit, cortisol, protein, glucose, lactate, and catecholamines) were measured and compared.

At T=60s, the glucose and catecholamine concentrations were significantly higher in group 2 than in group 1 (Bonferroni [Dunn] t test, $p < 0,05$). Since the degree of stress can be deduced from the levels of hormones during the stress response, we conclude that the use of the captive bolt stun is important in reducing the stress of the slaughter procedure.

Differentiation between NAD independent *Haemophilus paragallinarum* and *Ornithobacterium rhinotracheale* by polymerase chain reaction*

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Nicotine-adenine-dinucleotide (NAD) independent *H. paragallinarum* and *O. rhinotracheale* are two bacterial species causing upper respiratory tract infection in poultry. Both bacterial species appear very similar on BTA plates, while their biochemical reactions are also similar. It is thus very difficult to differentiate between *O. rhinotracheale* and NAD independent *H. paragallinarum* by conventional methods.

A polymerase chain reaction (PCR) test, which was developed for conventional *H. paragallinarum,* was tested as a rapid method to differentiate between these species. DNA from 30 different NAD independent *H. paragallinarum* and 30 different *O. rhinotracheale* isolates was isolated by heating at 120°C for ten minutes. The PCR was performed using 50 µl PCR Mastermix (Boerhinger), 2 µl each of *H. paragallinarum* specific primers, and 2 µl of the extracted DNA. The solution was made up to 100 µl with PCR grade sterile distilled water. The PCR was carried out in a Perkin Elmer 9600 thermal cycler at 35 cycles at 94°C for one minute, 65°C for one minute and 72°C for two minutes. Thereafter the PCR produce was run on a 0,7 % agarose gel, containing ethidium bromide, at 80 W for 30 minutes.

DNA amplification, as seen by a band of DNA larger than the primer band was found for all of the isolates biochemically identified as NAD independent *H. paragallinarum*, while no amplification of DNA was found in all of the samples biochemically identified as *O. rhinotracheale*.

It could thus be concluded that the PCR is specific not only for the normal NAD dependent *H. paragallinarum*, but also for the NAD independent *H. paragallinarum*. This PCR can also be used to differentiate between *H. paragallinarum* and *O. rhinotracheale*. Due to the speed at which PCR can be performed, specific identification of the causative agent can be obtained at least three days sooner then by conventional methods. This will allow correct and rapid treatment of the infection.

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Correlation between the ability to haemagglutinate red blood cells and sensitivity to Fosbac of *Ornithobacterium rhinotracheale**

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Ornithobacterium rhinotracheale is a bacterium which was first described from poultry in South Africa in 1990. Little is known about this organism which is difficult to differentiate from the nicotine-adenine-dinucleotide (NAD) independent variants of *H. paragallinarum*. Little is also known about serotypic and biochemical differences between the different isolates of *O. rhinotracheale*.

As part of a project to investigate the differentiation between *O. rhinotracheale* and NAD independent *H. paragallinarum*, and to study the diversity among *O. rhinotracheale* isolates, minimum inhibition concentrations (MIC) against Fosbac (25% fosfomycin) were investigated. A correlation between the sensitivity to Fosbac and other perceived biochemical and serological differences among *O. rhinotracheale* isolates was also investigated.

A total of 41 freeze-dried isolates were identified as either *O. rhinotracheale* or NAD independent *H. paragallinarum* based on carbohydrate fermentation patterns. The rate at which the different carbohydrates were fermented was noted. A suitable method for performing MIC's against *O. rhinotracheale*, in 96 well plates, was established and all of the isolates were tested using this method. Haemagglutination (HA) and haemagglutination inhibition (HI) tests were performed, using the methods standardized for *H. paragallinarum*.

Of the 25 isolates biochemically identified as *O. rhinotracheale*, 16 were found to haem-agglutinate chicken red blood cells (RBC). Of these, 11 isolates were found to have MIC values of below 128 μg/ml. All of the isolates which did not haemagglutinate the chicken RBC had MIC values of $\geq$ 128 μg/ml. The correlation between sensitivity to Fosbac and the ability to haem-agglutinate RBC was statistically significant. No correlation between the rate at which the carbohydrates were fermented, the sensitivity to Fosbac, or the ability to agglutinate RBC could be found. All the 16 NAD independent *H. para-gallinarum* isolates showed MIC values of between 4 - 8 μg/ml.

It was concluded that *O. rhinotracheale* isolates which did not possess the ability to agglutinate RBC were highly resistant to Fosbac. The antigens involved in haemagglutination may therefore play some role in the uptake of this antibiotic. If these antigens are absent, as seen by the inability to agglutinate RBC, the antibiotic cannot be taken up which results in the high level of resistance. The use of MIC against Fosbac could also be used as a method to differentiate between *O. rhinotracheale* and NAD independent *H. paragallinarum*.

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* *Research Project No. 36.5.221. Approved by the Faculty Ethics and Research Committees.*

Healthier animals mean healthier people*

Outreach Co-ordinating Committee

The Outreach Co-ordinating Committee aims to stimulate new programmes and build out the Faculty's involvement in community outreach.

The following programmes are in place:

1. A mobile ambulance providing a clinical service every two weeks to specific areas of the community of Hammanskraal, North West Province.

2. A Primary Veterinary Health Care service offered by preclinical students to schools in the Hammanskraal area.

3. Interactive Teleteaching whereby High School children in the Gauteng and North West Provinces receive instruction via an interactive television link. Programmes have included information on a career in veterinary science, as well as primary health care in animals. The Faculty also took part in a special Winter School programme for matriculants.

The committee is also involved in developing various research projects to address the community's needs. One such project investigated the socio-economic role of working donkeys in Hammanskraal and their helminth levels.

Visual material of the activities of the committee will be presented.

The members of the Outreach Co-ordinating Committee are: Prof. R.C. Krecek (convenor), Prof. R.I. Coubrough, Dr M. More O'Ferrall-Berndt, Dr C. Tutt, Dr N. Swanepoel and Dr P. Thompson.

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