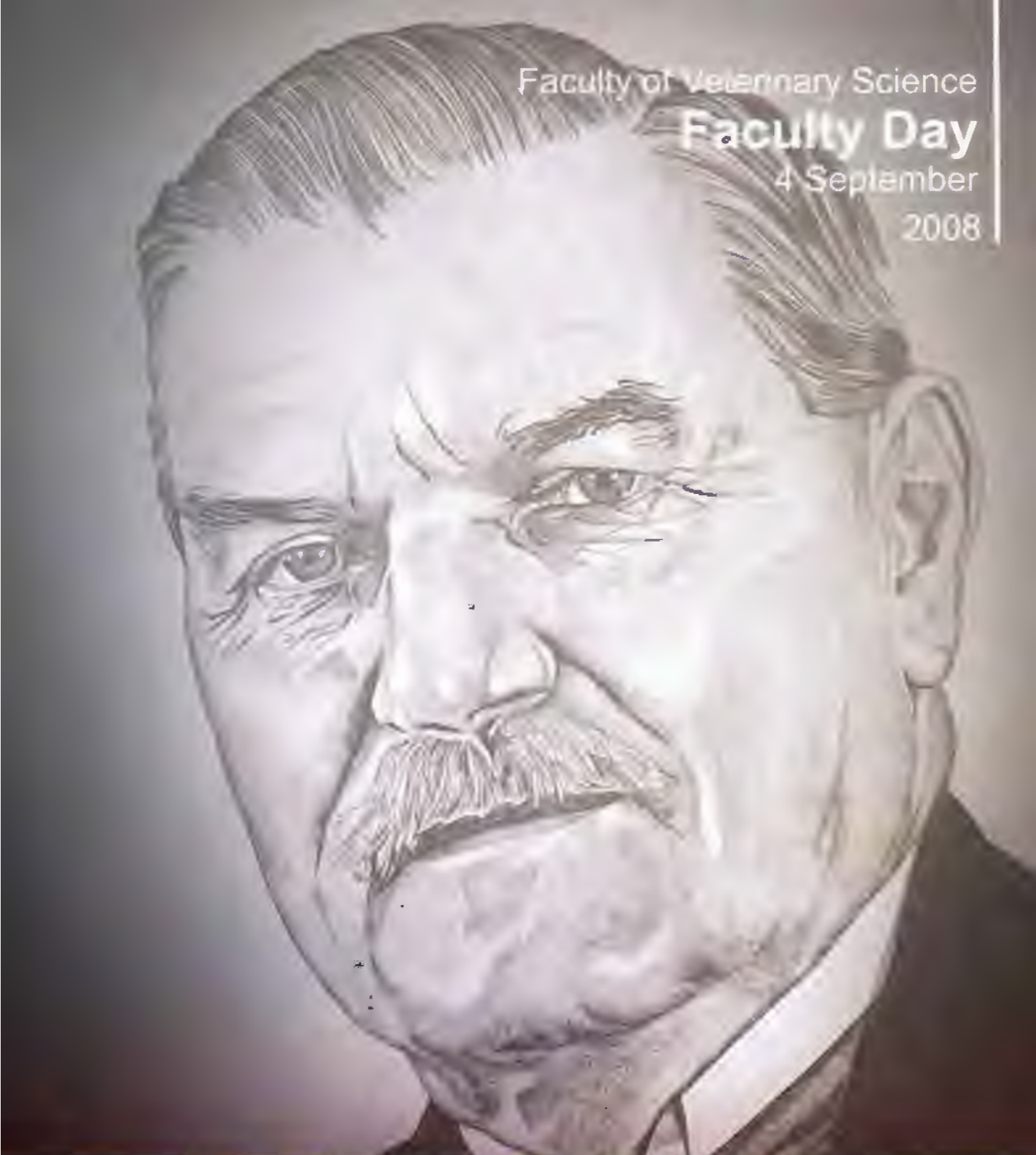
Faculty of Veterinary Science

Faculty Day

4 September

2008



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UNIVERSITEIT VAN PRETORIA
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Faculty of Veterinary Science

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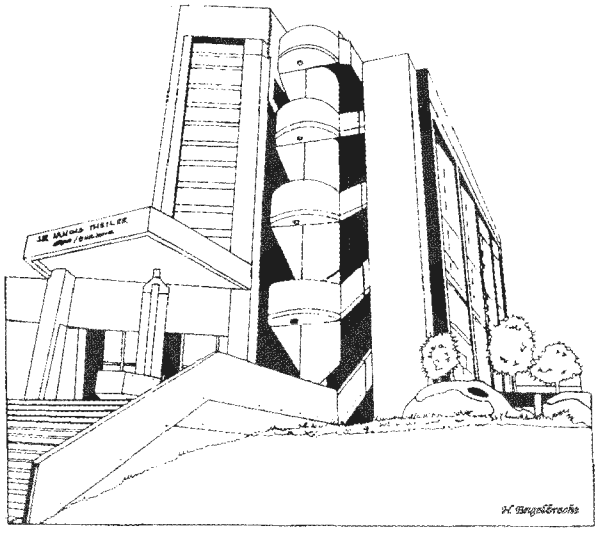
University of Pretoria

4 September 2008



Brief History of Faculty Day

Faculty Day of the amalgamated Faculty of Veterinary Science at the University of Pretoria reflects a proud tradition, which had been nurtured by the original Faculties of Veterinary Science of both Medunsa and the University of Pretoria, of advertising the research activities of staff and students on a special, dedicated occasion. Since the inception of the Faculty of Veterinary Science at Medunsa in the early 1980s, the staff, and later students, were involved in the activities of the "Academic Day" which aimed at highlighting the research activities of the University as well as exposing young researchers to a conference environment. The Faculty of Veterinary Science of the University of Pretoria at Onderstepoort followed this trend shortly thereafter and the first "Faculty Day", which focused on the research activities of the faculty, was held on 5 September 1984, sponsored by the then Dean, Prof JMW le Roux. The combined research skills of the two original institutions are today reflected in the proceedings of the Faculty Day held each year in the spring at the Onderstepoort campus

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Programme Design by Marté Smit

Sponsorships

The Faculty of Veterinary Science wishes to express its sincere thanks to the following sponsors for their very generous contribution towards the support of the 2008 Faculty Day Programme:

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Message from the Dean

The University of Pretoria is in the midst of its centenary celebration which makes Faculty Day this year even more special. It also coincides with the centenary year of the Onderstepoort Veterinary Institute from which veterinary training in South Africa had its origin in 1920. Veterinary Science has contributed significantly to the academic stature of the University of Pretoria in the early years of its development and continues to do so. As the only Faculty of Veterinary Science currently in South Africa it gives credibility to the notion of the University that it wishes to be locally relevant and having a local impact. As part of the Veterinary Research Institute, now OVI, the original Faculty of Veterinary Science became internationally renowned for outstanding research achievements in animal diseases and disease control.

These achievements are exemplified by the work of Sir Arnold Theiler, the first Dean of the Faculty of Veterinary Science of the Transvaal University College, now University of Pretoria, and Director of Veterinary Research and many other world famous veterinarians that followed him.

Faculty Day has formed the focal point of our academic year for some 24 years. It provides a forum for its staff and post graduate students to share the excitement of their research findings or experience with both student and colleague. Faculty Day has contributed significantly to our research growth over these years. The Sir Arnold Theiler Memorial Lecture remains a highlight of the proceedings presented by an esteemed veterinary scholar, scientist or researcher and in honour of the contribution made to the promotion of veterinary science and education. This year we honour Dr Gideon Brückner, Deputy Director of the World Organisation for Animal Health (OIE), a graduate of the Faculty, for his outstanding contribution to the promotion of veterinary science. It also celebrates the teaching and research achievements of our academic staff and award various undergraduate student prizes for performance excellence.

Welcome to Faculty Day and many thanks to the organisers and sponsors of the day. Your contributions and commitment are instrumental in making this day possible.

PROF GERRY SWAN
DEAN

Curriculum Vitae

Dr Gideon Brückner

Gideon Brückner was born in Cradock in the Eastern Cape and qualified as a veterinarian at the University of Pretoria (Onderstepoort) in 1972 and thereafter also obtained a B.Admin degree in Public Administration at the University of South Africa and a B.Admin (Hons) (cum laude) in International Administration at the University of Pretoria. He is married to Elsabé and has 3 children and 3 grandchildren. During his 34 years in the Government veterinary service of South Africa he worked as a state veterinarian in various localities in the country before he moved to the headquarters of Veterinary Services in Pretoria in 1981. He proceeded through the ranks and held the positions of Director of Veterinary Public Health, Director of Animal Health and Director of Veterinary Services in the National Department of Agriculture before he accepted the post of Chief Director of Veterinary Services in the Western Cape Department of Agriculture in 2001.

In February 2006 he accepted an offer from the World Organisation for Animal Health (OIE) to become Head of the Scientific and Technical Department of the OIE in Paris, France. In October 2007 he was promoted to Deputy Director General (Animal Health and International Trade) of the OIE.

Dr Brückner has spent his entire career as a veterinarian within provincial, national and international veterinary services. He was responsible for the management of several major animal disease outbreaks in South Africa such as foot and mouth disease, rabies, avian influenza, swine fever and Corridor disease. He has published 39 scientific articles of which 30 as senior author and contributed to chapters in two textbooks on animal diseases and post-graduate courses on animal disease control at the RCVS in the United Kingdom. He represented South Africa and the OIE on numerous missions and international conferences and served in an expert advisory capacity to international organisations such as the Food and Agricultural Orginasation (FAO), World Health Organisation (WHO) and the World Trade Organisation (WTO). He was President of the OIE Permanent Working Group on Animal Informatics and Epidemiology from 1990 to 2001. He chaired several ad hoc expert Groups of the OIE and was an elected member of the Scientific Commission for Animal Diseases of the OIE from 2003 to 2006. In 2005 he was honoured with the Presidents Award of the South African Veterinary Association.

Programme

08:00 to 08:30

Registration and Coffee

Master of Ceremonies: Dr CH Annandale

08:30 to 08:45

Welcome and Opening Address

Dean: Prof GE Swan

08:45 to 09:45

Research Programme: Oral Presentations I

Session Chairperson: *Prof H B Groenewald*

Canine paediatric gastrointestinal ultrasonography
N Stander, W M Wagner, A Goddard

The effect of respiratory phase on radiological tracheal dimensions in normal thoroughbred horses
A Carstens, R M Kirberger, R J Grimbeek

Morphology of the Emu (*Dromaius novaehollandiae*) tongue
M R Crole, J T Soley

Causes and clinical outcomes of after-hours equine admissions at a university referral hospital (1998-2007)
A Viljoen, M N Saulez, B Gummow, C M Donnellan, L Bester

09:45 to 10:30

Sir Arnold Theiler Memorial Lecture:

Dr G Brückner

"New challenges for the veterinary profession in global animal disease control and the trade in animals and animal products"

10:30 to 11:00

Awards Presentation

Lecturer of the Year
Young Lecturer of the Year
Nursing Lecturer of the Year
Researcher of the Year
Young Researcher of the Year
Prizes for academic achievements

11:00 to 11:45

Tea

Viewing of Posters, Commercial Exhibits and Photographic Exhibition

11:45 to 12:45

Research Programme: Oral Presentations II

Session Chairperson: *Dr M C Oosthuizen*

Identification of a novel *Theileria* species from African buffalo using 18S rRNA gene sequence analysis
M E Chaisi, K P Sibeko, M Troskie, N E Collins, M C Oosthuizen

Determining the genetic diversity of *Theileria equi* and *Babesia caballi* in Zebra (*Equus quagga* and *Equus zebra*)
R Bhoora, B L Penzhorn, A J Guthrie, N E Collins

Molecular identification of *Mycobacterium tuberculosis* complex in livestock and humans in Nigeria
A O Jenkins, L Streicher, S I B Cadmus, E H Venter, J Godfroid

A challenge study to determine the efficacy of Avinew Newcastle disease vaccine against challenge with goose paramyxovirus
D G Bwala, S P R Bisschop, A van Wyk

12:45 to 13:45

Light Lunch – Cafeteria

13:45 to 14:45

Research Programme: Oral Presentations III

Session Chairperson: *Prof M van Vuuren*

The *BrEMA1* gene: a tool for correlating *Babesia rossi* genotypes and clinical manifestation of canine babesiosis
P T Matjila, B Carcy, A L Leisewitz, T Schetters, F Jongejan, B L Penzhorn

C-reactive protein in canine babesiosis and its association with outcome
L S Köster, M Van Schoor, A Goddard, M Kjelgaard-Hansen, P N Thompson

Liver dysfunction rather than adrenal failure contributes to hypoglycaemia in canine babesiosis
J P Schoeman, P Rees, M E Herrtage

14:45 to 16:00

Research Programme: Presentation of Posters

Session Chairperson: *Prof E H Venter*

P1. **A novel *Babesia* species found in cheetahs (*Acinonyx jubatus*) in South Africa**
A-M Bosman, M C Oosthuizen, E H Venter, B L Penzhorn

P2. ***In vitro* and *in vivo* evaluation of five low molecular weight proteins of *E. ruminantium* as potential vaccine candidates for heartwater**
S I Sebatjane, A Pretorius, H Steyn, J Liebenberg, M Van Kleef

P3. **The evaluation of the susceptibility of *Trypanosoma congolense* isolates collected from cattle and buffalo in KwaZulu-Natal to isometamidium chloride and diminazene aceturate**
J F du Plessis, J Masumu, A R Nkuna, B L Penzhorn, P T Matjila, P van den Bossche

P4. **An update of the bovine trypanosomiases situation at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal Province, South Africa**
R Nkuna, J Esterhuizen, T Matjila, B L Penzhorn, S Geerts, T Marcotty, P van den Bossche

P5. **Molecular analysis of *M. bovis* isolated from buffaloes in Hluhluwe iMfolozi Park, South Africa**
A O Jenkins, L Streicher, E H Venter, D Cooper, J Godfroid

P6. **Genetic characterization of dog rabies viruses from Nigeria**
M F Ogo, L H Nel, C T Sabeta

P7. **Identification of a small *Babesia* species found in a dog imported from Taiwan**
M Troskie, N Mabogoane, O Matthee, B L Penzhorn

P8. **Determination of the minimum protective dose for bluetongue serotype 2, 4 and 8 vaccines in sheep**
J Modumo, E H Venter

P9. **Detection of feline coronavirus in cheetahs using real-time PCR**
A-M Bosman, M van Vuuren, J S Peck, J M Bernard, M Kennedy

P10. **Serum biochemistry changes in virulent canine babesiosis**
J P Schoeman, V McClure

P11. Admission serum biochemical parameters are not associated with mortality in puppies with parvo viral diarrhoea
J P Schoeman, M van Schoor

P12. Prevalence of and risk factors for feline hyperthyroidism in Hong Kong
C S de Wet, C T Mooney, P N Thompson, J P Schoeman

P13. Major outer membrane proteins of *Ehrlichia* species are mostly polymorphic and have evolved by gene duplication and genetic drift without positive selection pressure
B A Allsopp

P14. STR analysis: isolating DNA from semen smears on microscope slides
A Bierman, C Harper, M Schulman, A Nel, I Vorster

P15. Membrane specialisations in the efferent ducts of the ostrich epididymis
M Z J Elias, J T Soley, T A Aire, L du Plessis

P16. Admixture and founder origins in captive cheetahs (*Acinonyx jubatus*) detected using spatial Bayesian clustering
S P Sasidharan, C K Harper, A Ludwig, I Vorster, A Guthrie

16:00 to 16:30

TEA

Viewing of Posters, Commercial Exhibits and Photographic Exhibition

17:00

Cocktail Function & Prize Giving

During the cocktail function the following awards will be presented:
Best Scientific Paper; Best Scientific Poster; Photography prizes

The following exhibitions are on view in the foyer of the Sir Arnold Theiler Building throughout the day:

1. **Photographic Exhibition**
   An exhibition of photographs taken by staff and students. The submitted work will be judged by experienced photographers.

2. **Exhibits by Sponsors**

3. **Scientific Posters**

New challenges for the veterinary profession in global animal disease control and the trade in animals and animal products

Gideon Brückner (g.bruckner@oie.int)

Deputy Director General, World Organisation for Animal Health (OIE), 12 Rue de Prony, 75017, Paris, France

The Faculty of Veterinary Science of the University of Pretoria, is today one of the few veterinary faculties in the world that has the privilege to look back at 100 years of turmoil, essential changes and the dedication and achievements of its gallery of eminent researchers, teachers, academia and students who all contributed to earn this faculty its worldwide recognition as a veterinary institute of indisputable excellence. In doing so is also accepting that past successes provide solid evidence for what can be achieved in the future. However, the rapid changes in the global epidemiology of animal diseases do not offer the profession the luxury to ruminate on these past achievements, as the challenges facing the veterinary world today, pose in more than one way the challenges of the past 100 years compacted into one package that is already putting our skills, current perceptions, ability to change and ability to innovate, to the test and will increasingly do so in the next five to ten years.

Several warning lights were flashed to us during the past 10 to 15 years – not so much by the diseases themselves that occurred but by the circumstances under which they occurred, the underlying causes for their rapid global spread and the way in which the profession reacted. But also increasingly so – the intensive way in which the public at large and other professions and disciplines reacted to our way to deal or not to deal, with these events. The veterinary profession has been placed under public scrutiny as seldom before and in doing so, also demonstrating a global interest and awareness in the role of the profession in promoting animal health and its linkage to safeguarding human health. The World Organisation for Animal Health (OIE) in realizing this new focus on the delivery of veterinary services has through the initiative of its current Director General, Dr Bernard Vallat, urged the international community to accept the delivery of veterinary services as a global public good. While the profession is obviously thrilled by the growing international perception that the delivery of veterinary services, either public or private, is now increasingly accepted globally as a public good, it also calls for the realization of the implied obligation that global public goods are those whose benefits should in principle be enjoyed by the governments and peoples of all states. But also equally important, that operating in a world of global public good is also to accept operating in a world of shared risks and common opportunities grounded in the realities of mutual dependence and growing interconnection[1].

Accepting the challenge of operating in an environment of global public good, is also accepting the inevitable consequences and challenges of practicing a scientific discipline in an environment that will be questioned on non-scientific and very often also political grounds. It may have started with the BSE crisis in the late 1980's, but other events such as the foot and mouth disease outbreaks almost simultaneously in South Africa, the United Kingdom and Europe in 2000 and 2001; West Nile fever; SARS; NIPAH virus; the unprecedented rapid spread of highly pathogenic avian influenza over 4 continents in less than three years; classical swine fever in South Africa; African swine fever in Georgia and Azerbaijan; bluetongue in Europe and most recently the re-occurrence of Rift Valley fever in Africa and Madagascar. These are but a few examples warning us that events that were previously perceived as being exclusively of veterinary concern and responsibility are now also being claimed as the concern of politicians, the public at large and of many other disciplines that might sometimes have or not have an interest or a role to play. These incidents also indicate clearly that we should be sensitive to realities such as: that diseases now have the potential to spread across the globe faster than the average incubation period of a disease as there is no place in the world from which we are remote anymore or from whom we are disconnected; that 60 percent of human pathogens are zoonotic and 75 percent of emerging diseases are zoonotic; that 80 percent of agents

[1] International Task Force on Global Public Goods. 2006. *Meeting Global Challenges: International Cooperation in the National Interest.* Final report. Stockholm, Sweden.

having a potential bioterrorist or agri-terrorist potential are zoonotic pathogens; that the estimated annual illegal trade in animals is estimated at US$ 4 to 6 billion and that changes in human demographics and behavior and changed patterns of land use are contributing significantly to the spread of animal diseases and pathogens – the so-called ruralisation of the urban environment.

This calls for a different way in which we will have to look at diseases in the future: by looking for example beyond the classical post mortem lesions of a Rift Valley fever case and to also see the weather map of predicted climatic changes that preceded the outbreak or warned against new threats; by appreciating that an outbreak of anthrax in a dairy herd might also block the exports of products of other non-affected farms or herds; when detecting impurities in vaccines to also acknowledge that it might lessen the acceptance of guarantees to prove absence of disease and by failing to make a possible epidemiological link between migratory birds sharing meals with an ostrich herd could also lead to failure to explain an outbreak of avian influenza some months later. The international community is expecting the profession to look differently at the disease environment. They also expect that the responsibility for declaring an export safe for animal or human use – whether it is the live animal or a product of that animal – represents a scientific approach to surveillance by not only the certifying veterinarian but by every veterinarian that was in some stage of the production cycle, directly or indirectly involved.

Operating in a global public good environment and therefore also in an environment where there is a growing realization of the strong link between the occurrence of animal diseases and human health, has also resulted in a pressing and urgent identity and positioning challenge to the veterinary profession. In dealing with the global avian in-fluenza crisis, the OIE has maintained its position that the only sure way of preventing a human pandemic crisis of avian influenza, is to control and eradicate the disease at the animal source. This also holds true for most of the other zoonotic and emerging zoonotic diseases but with an important prerequisite that all countries whether developed, developing or transitional, should strengthen the ability of their veterinary services to move towards compliance with international sanitary standards and good veterinary governance. This challenge was also accepted by the international donor community in making available substantial funding to assist the OIE in assessing the performance of veterinary service delivery and their needs to move towards compliance with international sanitary standards. A total of 62 of the 172 OIE Member countries have been evaluated of which 31 of them in Africa alone. However, the realization of the close link between the control of animal diseases and especially human health; the increased importance of the animal/human/wildlife pathogen interface; the realization by the public of the critical role to be played by the veterinary profession and the realization by also the medical profession that control of these diseases as highlighted by the avian influenza crisis can best be achieved in a multi-disciplinary way, has resulted in increasing calls for support of the so called *One world, one health* concept. While this is an exciting and commendable development, the profession should approach this with an open and rational mind realizing the financial, constitutional and other related implications. But more importantly, to accept the need to maintain the identity, the role and the unique responsibility of the profession when consider taking hands with other professions in getting closer to a multidisciplinary approach to address the new exciting challenges to our profession.

I have more than sufficient reason to believe that the Faculty of Veterinary Science of this University is ready to take that challenge as it so excellently did for the past 100 years. I also believe that there is a full realization of the new responsibilities and also a sensitivity for old responsibilities that present themselves differently than in the past; that we will look with a new acknowledgement and realization if it is said that food security is also the responsibility of all veterinarians; that creating a buffer between the animal source of disease and human health is also our responsibility; that wildlife is no more the only responsibility of wildlife specialists or conser-va-tionists; that ensuring the safety of animal products in the shopping trolley of the consumer is no more only the responsibility of the veterinary public health veterinarian and lastly to appreciate that being accepted as deliverers of a global public good, is also to acknowledge that the obligation to remain in that environment, is to deliver the goods expected from us.

Canine paediatric gastrointestinal ultrasonography

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[2]Clinical Pathology Section, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

The normal ultrasonographic appearance of the adult canine gastrointestinal tract has been well described. The aim of this study was to document the normal ultrasonographic appearance of the gastrointestinal tract in puppies less than 12 weeks old. In addition, mesenteric lymph node size and appearance as well as assessment of any free abdominal fluid in normal puppies were also documented. These parameters are frequently examined when evaluating the presence of gastrointestinal disease.

Sixteen normal Beagle puppies from a research colony with no history of previous gastrointestinal disease were used in this study. The mean age of the puppies was 8.3 weeks (± 1.7 weeks SD) and mean weight 2.9 kg (± 0.4 kg SD). The animals were considered clinically normal based upon physical examination and laboratory evaluation. They were starved for 16 hours prior to ultrasonographic examination. Stomach, jejunal, duodenal and colonic wall thickness were measured ultrasonographically. Additionally duodenal and jejunal mucosal width were also measured. The number of peristaltic contractions per minute was recorded for each bowel segment if possible. The amount and echogenicity of any free abdominal fluid was noted. The number, echogenicity and maximum thickness of the mesenteric lymph nodes was documented. Direct comparisons between the duodenal, jejunal and colonic walls as well as the duodenal and jejunal mucosal thicknesses were made by means of paired t-tests. Significance was set at $P < 0.05$. Mean, standard deviation and range were calculated for each variable measured. Linear regression models were used to assess the affect of puppy age and weight on each of the variables.

In all puppies, one to five mesenteric lymph nodes could easily be visualised. They were hypoechoic to the surrounding tissue and their mean thickness was 7.5mm (± 2.0mm SD). A mild amount of anechoic free abdominal fluid was observed in all puppies. The duodenal wall was significantly thicker than that of the jejunum ($P < 0.0001$); similarly the duodenal mucosal thickness was significantly thicker than the jejunal mucosal thickness ($P < 0.0001$). The jejunal and duodenal walls were statistically thicker than the colonic wall ($P < 0.0001$ respectively). There was a significant effect of puppy age on duodenal and stomach wall thickness ($P=0.042$ and $P=0.045$ respectively). There was no peristaltic activity observed in 10 of the 16 puppies.

To date, there are no published normal reference ranges for animals of this age or weight however changes in paediatric gastrointestinal wall thickness from the stomach to the colon follow similar trends to adult canines. Prominent mesenteric lymph nodes and a mild amount of anechoic free abdominal fluid can be normal findings in puppies.

Anatomical Site	Mean ± SD [mm]	Range ± SD [mm]	Total number measured
Stomach wall	2.8 ± 0.5	1.9 - 3.7	14/16 (88%)
Duodenal wall	4.0 ± 0.5	2.9 - 5.0	16/16 (100%)
Duodenal mucosa	2.8 ± 0.4	1.9 - 3.7	16/16 (100%)
Jejunal wall	2.5 ± 0.5	1.4 - 3.5	16/16 (100%)
Jejunal mucosa	1.5 ± 0.4	0.7 - 2.3	16/16 (100%)
Colon wall	1.4 ± 0.2	0.9 -1.8	14/16 (88%)

The effect of respiratory phase on radiological tracheal dimensions in normal Thoroughbred horses

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[2]Department of Statistics, University of Pretoria, South Africa

Respiratory conditions causing poor performance in horses are usually as result of upper respiratory diseases (URT) or of pulmonary origin. The trachea, being the conduit between these two areas, is often overlooked, since it is rarely a cause of primary respiratory problems in the horse. Tracheal dimensions, particularly height (TrH), however, may be useful in recognizing problems in the URT and lungs due to the differential pressures occurring in the URT and lungs with pathology. Nothing on the normal tracheal dimensions during expiration (E) and inspiration (I) in the normal horse, has been published.

Standing lateral radiographs of the cervical and thoracic trachea of 15 Thoroughbred horses (TH), three to six years old, with clinically normal respiratory systems, were made at peak inspiration and end expiration. Maximum height of the larynx (LH), trachea at the level of the third cervical vertebra (TrHC3), trachea at C5 (TrHC5), trachea at the thoracic inlet (TrHTI), the carina (CarH) and the left and right primary bronchi (LBrH and RBrH) were measured. Ratios of LH and TH relative to adjacent vertebral body lengths (VBL) were made for normalization. Known size metallic markers were used to determine magnification-corrected (MfC) tracheal heights in the sagittal plane and effect of body mass and height at the withers on TH.

The table shows the mean values of airway height (cm), with no significant differences between expiration and inspiration seen. No effect of body mass or height on TH were seen in this study, contrary to previously published findings where mass of different mammalian species was related to TH, although the horse masses in this study had a small range, from 421 to 559 kg.[1]

There is little radiological difference in tracheal height of the Thoroughbred horse at inspiration and expiration. This study in normal horses may serve as a reference when radiologically evaluating cases such as recurrent airway obstruction or URT disease, where these dimensions may differ significantly due to differences in airway resistance and biomechanics. This is part of a larger study where other parameters of thoracic radiographs in these horses is being evaluated.

References

1. Leith D E 1983 Mammalian tracheal dimensions: scaling and physiology. *Journal of Applied Physiology* 55:196-200.

	LH		TrHC3		TrHC5		TrHT1		CarH		LBrH		RBrH	
	E	I	E	I	E	I	E	I	E	I	E	I	E	I
Non-MfC	7.7	7.6	5.0	5.3	4.8	4.7	4.6	4.5	6.3	6.3	4.6	4.4	5.9	6.0
MfC	-	-	4.2	4.0	3.6	3.6	3.4	3.2	3.9	4.1	-	-	-	-
LH – TrH height/ VB length	0.6	0. 6	0.4	0.4	0.4	0.4	0.6	0.6	1	0.0	-	-	-	-

Morphology of the Emu (*Dromaius novaehollandiae*) tongue

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Previous studies would seem to suggest that the rudimentary tongue of ratites has a limited function, namely, to depress the floor of the oral cavity during feeding. Reports on the morphology of the tongue are sketchy and limited to descriptions of its shape and superficial appearance. The aim of this study was to document the morphological features of the tongue of the emu in order to better understand its function.

The heads of twenty 14-15 month-old emus obtained from a local abattoir were immersion-fixed in buffered formalin for at least 48 hours. All the heads were used for macroscopic descriptions of the tongue. Three tongues were dissected to reveal the lingual skeleton and hyobranchial apparatus. Five tongues were cut into longitudinal and transverse sections and routinely processed for light microscopy. H & E and PAS-stained sections were examined, described and digitally recorded.

The tongue was dorso-ventrally flattened and triangular or dagger-shaped, with the apex pointing rostrally. The surface was pigmented and appeared grey/brown in formalin-fixed specimens. The lateral margins displayed a variable number of caudo-laterally directed papillae which progressively became longer and more slender from the apex caudally, giving the lateral margins a serrated appearance. The caudal margin revealed 1-4 caudal papillae. A triangular, connective tissue frenulum attached the ventrum of the tongue to the floor of the oral cavity. The root of the tongue appeared to be represented by a triangular structure situated at the laryngeal entrance and lying within the arms of the *Ceratobranchiale*. The apex of this structure (facing the laryngeal entrance) displayed a raised, rounded, sometimes pigmented, projection. The *Paraglossum* (dorsally) and the rostral projection of the *Basihyale* (ventrally) formed the lingual skeleton.

The tongue was covered by a lightly keratinised (dorsally) and non-keratinised (ventrally) stratified squamous epithelium. Pigment-containing dendritic cells, mainly confined to the *stratum basale*, were only present in the dorsal epithelium. Pigment was also present in the underlying connective tissue, mainly associated with blood vessels. The most prominent feature of the underlying connective tissue was the presence of large, simple branched tubulo-alveolar, mucous-secreting glands. Ciliated columnar epithelium was occasionally seen to line the secretory duct of the glands. Isolated structures resembling taste buds were infrequently seen in the epithelium of the ventrum and tongue root, and were associated with the mucous gland openings. Numerous Herbst (Pacinian) corpuscles were closely associated with the glandular tissue. Diffuse lymphoid tissue occurred between the glands on the ventrum and was organised as nodules at the tip of the frenulum.

It was evident from this study that the function of the emu tongue is not restricted to depression of the mouth floor when swallowing (by virtue of its association with the hyobranchial apparatus). The ubiquitous mucous glands would provide lubrication and protection, whereas the lymphatic tissue would fulfil an immunological function. The numerous pressure receptors (Herbst corpuscles) could assist in distinguishing the type or texture of food to be ingested as well as its correct positioning for swallowing, and the taste receptors may assist in food selection.

Causes and clinical outcomes of after-hours equine admissions at a university referral hospital (1998-2007)

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The aim of this study was to determine the reasons for admission, clinical findings, length of hospitalization, cost and outcome in horses admitted after-hours as an emergency to the Equine Clinic of the Onderstepoort Veterinary Academic Hospital over a 10-year period.

The medical records of all after-hours equine admissions during 1998 to 2007 were reviewed and classified as either a true emergency or not. Data extracted from the medical records included age, sex, breed, reason for admission, pre-admission treatment, clinical presentation, procedures performed, final diagnoses, complications occurring in hospital, length of stay, cost of hospitalization and outcome.

Of the 1136 after-hours admissions, 820 records were available and 75% of admissions were classified as true emergencies. Most horses originated from the Gauteng province (76%) with Thoroughbred, Arabian, and Warmbloods representing 47%, 12% and 7% of horses. Horses had a median age of 7 years and were predominantly male (62%). Gastro-intestinal (49%) and musculoskeletal (28%) related disorders were the primary reasons for admissions. Most horses received anti-inflammatories (64%), sedation (23%) and antibiotics (21%) before admission. On admission, the majority of horses had medical intervention (76%) while surgical intervention was performed in 17% of horses. Intravenous catheterization (48%), rectal examination (46%), nasogastric intubation (42%), abdominocentesis (24%) and ultrasonography (14%) were the procedures performed most frequently. Surgical and medical colics constituted 21% and 20% respectively of the overall diagnoses confirmed in the hospital, while babesiosis was diagnosed in 4% of horses. Post-admission complications occurred in <2% of horses. The median length of stay and cost of hospitalization was 5 days (95% CI: 1 to 23 days) and ZAR 2,007 (95% CI: 72 to 12,325 ZAR) respectively. Eighty one percent of all horses admitted after-hours survived to discharge, while 16% were euthanased and 4% died.

This study demonstrated that the majority of after-hours equine admissions to a university referral hospital required medical inter-vention and were mostly due to gastro-intestinal related disorders. Overall, the survival rate for after-hours admissions was good. This study may assist in the after-hours emergency planning and training of personnel.

Identification of a novel *Theileria* species from African buffalo using 18S rRNA gene sequence analysis

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The African buffalo (*Syncerus caffer*) is the natural reservoir host of both pathogenic and non-pathogenic *Theileria* species. Corridor disease, caused by *Theileria parva,* is a controlled disease in South Africa. Buffalo are the natural reservoir host of this parasite, which is transmitted by *Rhipicephalus appendiculatus* and *R. zambeziensis*. Buffalo also appear to be the original hosts of two other *Theileria* species infecting cattle, the relatively benign *T. mutans* and the apathogenic *T. velifera*, both of which are transmitted by *Amblyomma hebraeum*. *Theileria buffeli* and the hitherto uncha-racterized *Theileria sp.* (buffalo) have thus far only been identified in some buffalo populations in South Africa[1]. Very little is known about *Theileria* sp. (buffalo) which was first recognised in an isolate from a buffalo in Kenya[2]. *Theileria* parasites usually occur as mixed infections and although the benign and non-pathogenic forms do not have any significant economic importance, they can interfere with the diagnosis of the pathogenic forms (e.g. *T. parva* in South Africa) and therefore confuse their epidemiology.

Recently, buffalo blood samples (n=198) originating from the Kruger National Park and the Hluhluwe iMfolozi Park were screened for *Theileria* species using the Reverse Line Blot hybridization (RLB) assay. The RLB results demonstrated the presence of *T. parva*, *T. mutans*, *T. velifera*, *T. buffeli* and *Theileria* (sp.) buffalo, either as single infections or as mixed infections. In a number of samples the PCR products did not hybridize with any of the *Babesia* or *Theileria* species-specific probes present, only with the *Babesia/Theileria* genus-specific probe suggesting the presence of a novel species or variant of a species. Full-length 18S rDNA of two of these were amplified, cloned and the recombinants were sequenced. Sequencing data were analysed with the Staden package, aligned with published sequences of related genera using ClustalX and a phylogenetic tree was constructed using neighbour-joining in combination with the bootstrap method.

Sequence similarity analysis indicated that a *Theileria* species infection was present showing highest similarity (~98%) with *T. mutans*. A species-specific RLB oligo-nucleotide probe was designed in the hypervariable V4 region of the 18S rRNA gene and will be used to screen buffalo samples to determine the prevalence of this parasite in buffalo in South Africa.

References

1. Stoltsz W H 1996 Theileriosis in the African Buffalo. *Proceedings of a Symposium on the African Buffalo as a Game Ranch Animal* Onderstepoort, South Africa.
2. Allsopp B A, Baylis H A, Allsopp M T E, Cavalier-Smith T, Bishop R P, Carrington D M, Sohanpal B, Spooner P 1993 Discrimination between six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal DNA sequences. *Parasitology* 107: 157-165.

Determining the genetic diversity of *Theileria equi* and *Babesia caballi* in Zebra (*Equus quagga* and *Equus zebra*)

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Equine piroplasmosis is caused by two intraerythrocytic protozoal parasites, *Theileria equi* and *Babesia caballi*. Both parasites are tick-transmitted and infect horses, mules, donkeys as well as zebras. In a previous study on the characterization of the 18S rRNA gene of *T. equi* and *B. caballi* in horses, we found that the nucleotide sequences of two zebra samples from the Bontebok National Park in the Western Cape, showed a high degree of similarity to a previously published *T. equi*-like sequence. This genotype was not detected in any of the South African isolates from horses, suggesting that there is more variation in *T. equi* genotypes in zebra in South Africa. A study was thus undertaken to determine the level of genetic variation in the 18S rRNA genes of zebras infected with *T. equi* and/or *B. caballi*.

Seventy blood samples in EDTA, collected from Plains zebra (*Equus quagga*) and Mountain zebra (*Equus zebra*), were obtained from the Kruger National Park (n=20), the Wildlife Breeding Research Centre (n=14), and the Equine Research Centre (n=36). Samples were screened for the presence of parasite DNA using the reverse line blot hybridization (RLB) assay, and a *B. caballi*-specific TaqMan MBG real-time PCR test was used to confirm the presence of this parasite. The V4 hypervariable region of the 18S rRNA gene from the positive samples was amplified, cloned and sequenced. Phylogenetic relationships were determined using distance, maximum parsimony and Bayesian inference methods.

Reverse line blot hybridization (RLB) results indicated that 45 samples were negative for the presence of piroplasms. Seventeen samples were positive for *T. equi*, while no samples hybridized to the *B. caballi* probe. Eight samples hybridized to the *Theileria/Babesia* genus-specific probe only and not to any of the species-specific probes. Thirteen of the 17 zebra samples that were *T. equi* positive, also hybridized to the *Babesia* genus-specific probe but not to the *B. caballi* species-specific probe. These results indicate either a possible mixed infection with *B. caballi* at a level below the detection limit of the *B. caballi* RLB probe, or the presence of a novel *Babesia* species or genotype. However, a real-time PCR assay specific for the detection of *B. caballi* infections, was able to detect *B. caballi* parasite DNA in fourteen of the 25 RLB positive samples. Nucleotide sequence data was obtained from 15 of the positive samples and BLASTN analysis revealed that the sequences were most similar to *T. equi* genotypes and not *B. caballi* genotypes. Although *Babesia* parasites were present in some of these samples, the parasitaemia may have been too low to allow detection by cloning of PCR products from a mixed infection.

Based on these findings and our previous results obtained for isolates from horses, we conclude that there are three groups of *T. equi* 18S rRNA genotypes in South Africa. One genotype has only been isolated from horses in South Africa, another genotype has thus far only been identified in zebra in South Africa, while the third genotype is present in both horses and zebra.

Molecular identification of *Mycobacterium tuberculosis* complex in livestock and humans in Nigeria

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The true picture of the prevalence of tuberculosis and zoonotic tuberculosis in Nigeria is not accurately presented. One of the reasons is that the traditional techniques currently use for the diagnosis and the identification of the members of the *Mycobacterium tuberculosis* complex (MTBC), are not sensitive enough. This study aims at highlighting the relevance of molecular based tools in the identification of mycobacterial isolates obtained from cattle and humans with diverse risk factors in Ibadan, southwestern Nigeria.

Samples were obtained from organs of livestock after slaughter, patients diagnosed with pulmonary tuberculosis, extrapulmonary tuberculosis and HIV, as well as feces from infants, and cattle traders. Samples were processed using the Becton Dickinson procedure; inoculated onto Lowenstein-Jensen media with pyruvate and glycerol and incubated at 37°C for 8-12 weeks. DNA was obtained from culture positive isolates by boiling at 80 °C for 30 mins. DNA was then screened by a multiplex PCR that amplified the RD4 and RD9 regions. Further screening was done by amplifying the $RD1^{mic}$ and $RD2^{seal}$ regions using previously published primers. Spoligotyping was also done on these samples as described by Kamerbeek *et al.*, (1998). Sequencing data obtained were analysed using Bioedit and Mega 4.0 for sequence alignments and blast using the BLASTN.

After RD4 and RD9 deletion analysis performed on 53 cattle, 12 pig, 6 goat and 141 human isolates, 5 cattle and 2 human isolates were "classical" *M. bovis* (RD4 and RD9 deleted) and 30 human isolates were *M. tuberculosis* (RD9 and RD4 present). The $RD1^{mic}$ and $RD2^{seal}$ deletion analysis revealed *M. africanum* ($RD1^{mic}$ present; no amplification at $RD2^{seal}$) in 1 pig, 3 cattle and 20 human isolates. Forty one cattle, 35 human, 11 pig and 5 goat isolates could not be further described (presence of $RD1^{mic}$, $RD2^{seal}$ and RD4). Sequencing analysis of the $RD2^{seal}$ band in isolates from cattle and humans resulted in identical nucleotide sequences aligning with several *M. tuberculosis* and *M. bovis* sequences. Spoligotyping confirmed these unique isolates as *M. bovis* (absence of spacers 3, 9, 16, 39-43) on a panel of representative isolates; furthermore, an undescribed *M. bovis* spoligopattern (www.Mbovis.org and SpolDb4 database) was identified.

These results indicate that molecular tools are useful in presenting a clear picture of the array of species responsible for tuberculosis infections in humans and animals. Routine application of molecular techniques in clinical and research settings is therefore recommended, especially in a tuberculosis endemic country like Nigeria.

A challenge study to determine the efficacy of Avinew Newcastle disease vaccine against challenge with goose paramyxovirus

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A velogenic viscerotropic strain of Newcastle disease virus (NDV) known as "goose paramyxovirus" (GPMV) and belonging to the genotype VIId is reported to have been introduced into South Africa in about 2000. Since then GPMV has caused numerous outbreaks of Newcastle disease among both commercial and backyard poultry in South Africa. The disease is characterized by high morbidity and mortality resulting in massive economic loss to the poultry industry. The virus has also proved to be more persistent than previous strains and is known to cause disease in waterfowl which previous strains of NDV were not known to do. Control of the disease has proved difficult with vaccines appearing not to be completely effective in controlling the disease in the field, thus raising concerns regarding the ability of commercially available vaccines to protect flocks against this strain. The aim of this trial was to determine if there are differrences in the level of protection offered by Avinew Newcastle disease vaccine against challenge with GPMV (ICPI of 1.85) as compared to another velogenic strain of Newcastle disease isolated from a previous outbreak in South Africa in 1995 known as the "Rainbow challenge virus" (ICPI of 2.0 and genotype VIII).

Six groups of eighteen specific pathogen free (SPF) chickens were hatched, raised in isolation units and then vaccinated with doses of $10^{3.0}$, $10^{4.5}$ and $10^{6.0}EID_{50}$ of Avinew vaccine at 10 days of age. Two extra groups of eight SPF chickens served as unvaccinated controls. All the chickens were then challenged at 4 weeks of age intramuscularly with GPMV and "Rainbow challenge virus" (RCV) at a dose rate of 0.2 ml, allowing for a total dose of $10^{5.3}EID_{50}$ per bird, for both the viruses. Birds were observed twice daily and their clinical status scored as either 0 (normal), 1 (sick) or 2 (dead). Dead and euthanized birds were necropsied and gross pathological lesions observed and recorded. Data was subjected to statistical analysis and the protective dose (PD_{50} and PD_{90}) of the vaccine against challenge by both the viruses were calculated.

No significant difference could be found in the protection offered by Avinew vaccine against GPMV challenge and RCV challenge. At the recommended dose of $10^{6.0}EID_{50}$ the vaccine gave almost 100% protection against both the challenge viruses. The PD_{90} of Avinew against GPMV was calculated at $10^{4.38}$ versus that of RCV at $10^{4.43}$. This difference was not found to be statistically significant. Overall mortalities were slightly higher for the group challenged with the Rainbow virus than with the GPMV. The higher number of mortalities and the higher PD_{90} values for the groups challenged with Rainbow virus confirms the slightly higher pathogenicity of the Rainbow virus as earlier indicated by the ICPI values. However, vaccination with Avinew ND vaccine even at $10^{6.0}$ EID_{50} (optimal recommended field dose) was only able to protect against clinical disease and mortality but not against infection and replication of the viruses; as some degree of gross lesions were evident even in apparently healthy birds that survived the challenge without any clinical signs. Analysis of pathological lesions showed that more organs were affected in groups challenged with GPMV than groups challenged with RCV. This data was however not subjected to statistical analysis.

The *BrEMA1* gene: a tool for correlating *Babesia rossi* genotypes and clinical manifestation of canine babesiosis

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Babesia rossi, an intra-erythrocytic protozoan, causes a severe, often life-threatening, disease of domestic dogs. Dogs treated early for *B. rossi* infection usually recover from the disease, but dogs left untreated or treated at a later stage of infection seldom survive. Dogs infected with *B. rossi* have varied clinical manifestations that can be categorised as uncomplicated (with a good prognosis) or complicated (with a poor prognosis). *Babesia rossi* isolates have previously been identified by typing of a polymorphic repetitive region from the gene *Babesia rossi* Erythrocyte Membrane Antigen (*BrEMA1*).

Blood samples (n = 121) were collected from dogs presented to the Onderstepoort Veterinary Academic Hospital (OVAH) and diagnosed with babesiosis on thin blood smear. A further 20 samples were obtained from private clinics around the Onderstepoort, Johannesburg, Durban, White River and Cape Town areas. DNA was extracted from 200 µl of each blood sample using the QIAmp blood and tissue extraction kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. As a first step for the molecular diagnosis of *Babesia* species infecting each dog, a PCR was performed with Reverse Line Blot (RLB) primers F2 and R2. The *B. rossi* genetic diversity was then analysed on genomic DNA from samples that tested positive for *B. rossi* on the RLB. The samples were screened by PCR targeting the *BrEMA1* gene and sequencing of the polymorphic region (i.e. a variable number of hexapeptide repeats). Sequence data for the full *BrEMA*1 sequences were assembled using the GAP 4 of the Staden package. Sequence alignments were manually edited using Bioedit. DNA sequences were translated into amino acid sequences and genotyping was done according to the number of hexapeptide repeats. Sequence alignment of the amino acid sequences and phylogenetic trees were generated. The Cluster and Topological algorithm methods were used for the construction of phylogenetic trees into the phylogram format from the alignment sequences. Genotype frequencies were compared using a two-tailed binomial test. Proportions were compared using a two-tailed Fisher's exact test. Analyses were done using Stata 8.2.

Analysis of PCR products revealed 11 different gene profiles, visualised by gel electrophoresis. Twelve distinct *BrEMA*1 genotypes were identified by sequencing in which the numbers of hexapeptide repeats varied from 6 to 31 (classified as genotype6 to genotype31). The genotypes were compared to the case data. The most prevalent *B. rossi* parasites were those attributed to genotype19 (36.2%), genotype28 and 29 (20.6% each) and genotype11 (12.7%). These genotypes were also associated with poorest prognosis. Our preliminary findings reveal clinically important differences between the various *B. rossi* genotypes identified.

C-reactive protein in canine babesiosis and its association with outcome

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C-Reactive protein (CRP) is a major acute phase protein (APP) in dogs and identified as a useful predictor of outcome and severity in some diseases. *Babesia canis rossi* is a common infectious disease accounting for 10-12 % of the total caseload of sick patients seen in South African veterinary practices, of which 26% are considered complicated.

The aim of this study was to assess whether CRP concentration is an objective way of assessing severity of disease in dogs infected with *B. rossi*, particularly with regard to outcome.

A prospective study in dogs diagnosed with babesiosis and admitted to the Onderstepoort Veterinary Academic Hospital was undertaken. Diagnosis and babesia subtype were confirmed as *B. rossi* by PCR and Reverse Line Blotting. Exclusion criteria included severe intercurrent disease and euthanasia due to reasons other than poor prognosis. CRP concentrations were determined at presentation using an automated heterologous assay validated for use in dogs. The relationship between CRP concentration and outcome was analysed using exact logistic regression, adjusting forage and sex. Significance was set at $P \leq 0.05$.

Results of 75 dogs were available. Sixty eight dogs met the inclusion criteria, of which 57 dogs survived. Exact logistic regression analysis showed that the CRP concentration at admission was not predictive of outcome (P=0.53). The mean ± SD CRP concentration on admission for survivors (n=57) was 107.5 ± 49.5 mg/L and for non-survivors (n=11), 122.1 ± 64.6 mg/L.

There was no statistically significant differrence in CRP concentration between survivors and non-survivors. APPs are expected to be predictors of poor outcome, with a correlation between inadequate pro-inflammatory response associated with poor outcome. This does not seem to be the case in canine *B. rossi* infection in dogs, most likely due to the development of the fulminant form of the disease developing peracutely, with little opportunity of investigating kinetics of CRP concentration in the non-survivors. Canine CRP therefore cannot be considered a prognostic marker of outcome in canine babesiosis.

Liver dysfunction rather than adrenal failure contributes to hypoglycaemia in canine babesiosis

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Hypoglycaemia has been identified as a life-threatening metabolic complication in humans with malaria and in severely ill dogs suffering from *Babesia rossi* babesiosis. Hyperinsulinaemia, although demonstrated in human malaria, has been discounted as a potential cause in dogs. Other causes of hypoglycaemia may include adrenal failure and hepatic dysfunction.

This prospective, cross-sectional study, including 36 dogs with naturally occuring virulent babesiosis, sought to investigate the role of the liver and the adrenal gland in the pathophysiology of hypoglycaemia. The diagnosis of canine babesiosis was made on stained thin capillary blood smears. Pre-treatment jugular blood samples were collected for simultaneous determination of plasma glucose and serum cortisol, hepatic enzymes, and bile acid concentrations. Haematocrit was also measured. Patients were retrospectively divided into three groups: hypoglycaemic (blood glucose (BG) < 3.3 mmol/ℓ; n=12), normoglycaemic (BG 3.3-5.5 mmol/ℓ; n=12), and hyperglycaemic (BG > 5.5 mmol/ℓ; n=12). Statistical analysis was performed using the Kruskal Wallis test.

Data is expressed as median and inter-quartile range for the three groups, respectively: cortisol (nmol/ℓ) 371 (159-522), 122 (73-274), 103 (61-167); bile acids (µmol/ℓ) 60 (37-82), 30 (23-66), 27 (25-35); Alt (IU/ℓ) 171(78.3 344), 33 (273-381), 29 (245-400); Alp (IU/ℓ) 158 (99-236), 94 (56-237), 72 (147-260); Haematocrit (ℓ/ℓ) 0.09 (0.09-0.12), 0.27 (0.14-0.40), 0.13 (0.07-0.21). Median serum cortisol, bile acids, Alt and Alp concentrations were signifycantly higher ($p < 0.05$ for all) in the hypoglycaemic group than in the other blood glucose groups. Haematocrit was significantly ($p = 0.02$) higher in the normoglycaemic group and its similar reduction in the other two groups makes anaemia an unlikely sole cause of elevated Alt in the hypoglycaemic cases.

Hepatic dysfunction (elevated fasting serum bile acids) and increased hepatocellular permeability (elevated Alt) were identified in the hypoglycaemic cases. Elevated cortisol could result in Alp induction in hypoglycaemic cases, whereas hepatic dysfunction may contribute to decreased cortisol breakdown. Cortisol is an important counterregulatory hormone, and higher concentrations are appropriate in hypoglycaemia. We conclude that hepatic dysfunction, and not adrenal failure, is a contributory factor to hypoglycaemia in canine babesiosis. Parasite glucose consumption and depletion of hepatic glycogen stores are also likely to play a role in the pathophysiology of hypoglycaemia in canine babesiosis. Further studies are required.

A novel *Babesia* species found in cheetahs (*Acinonyx jubatus*) in South Africa

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The cheetah (*Acinonyx jubatus*) is listed in Appendix I (most threatened species) in the World Conservation Union (IUCN) Red List of Threatened Species (2008). Only 12,000 to 15,000 cheetahs remain in the wild, mostly in small, isolated populations in 24 to 26 countries in Africa, mainly because of loss of habitat in the wild and conflicts with farmers in remaining habitats. Cheetahs are also suspected to be particularly vulnerable to infectious diseases. Both small and large intraerythrocytic piroplasms have been reported from a variety of domestic and wild feline species from several continents. Little is known about the prevalence of these piroplasms in cheetahs, however. The aim of the current study was to characterize these undescribed *Babesia* spp. by phylogenetic analysis based on the 18S rRNA gene and the second internal transcribed spacer region (ITS-2) sequences.

Thirteen EDTA blood specimens, previously collected from captive cheetahs and screened with the reverse line blot (RLB) hybridization assay, were selected for this study[1]. PCR amplicons hybridized only with the *Babesia* genus-specific probe, which indicated the presence of a novel species or variant of a species. The near full-length 18S rRNA gene (~1700 bp) and ITS-2 gene (~600 bp) were amplified and sequenced. Sequence data were assembled and edited using GAP 4 of the Staden package (Version 1.6.0 for Windows). The assembled sequences were aligned with sequences of related genera using ClustalX (Version 1.81 for Windows). Phylogenetic trees were constructed using neighbor-joining and the maximum parsimony methods.

A BLAST search performed with the obtained 18S rRNA gene sequences revealed no identical sequences in the public databases and the new sequences were designated *Babesia* sp. (cheetah). The most closely related sequence (~96% identity) was from *Babesia conradae* (AF231350 and AF158702), previously identified from dogs in California, USA. Similar results were obtained with sequencing data from the ITS-2 gene which confirmed the presence of new *Babesia* species in cheetahs.

Reference

1. Bosman A-M, Venter E H, Penzhorn B L 2007 Occurrence of *Babesia felis* and *Babesia leo* in various wild felid species and domestic cats in Southern Africa, based on reverse line blot analysis. *Veterinary Parasitology* 144: 33-38.

In vitro and *in vivo* evaluation of five low molecular weight proteins of *E. ruminantium* as potential vaccine candidates for heartwater

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Heartwater is a tick-borne disease of domestic and some wild ruminants caused by *E. ruminantium*. Cell mediated immune responses accompanied by IFN-γ production are required for protection against heartwater and proteins that can induce such responses could be good vaccine candidates. Previously low molecular weight proteins of *E. ruminantium* were shown to induce cellular immune responses characterized by proliferation of immune PBMC, proliferation of $CD4^+$-enriched T-cells as well as production of IFN-γ by these cells.

In an attempt to develop a DNA vaccine for heartwater, potential vaccine candidates were identified from *E. ruminantium* genome using Bioinformatic tools targeting genes encoding low molecular weight proteins. Five open reading frames (ORFs) were identified and their corresponding recombinant proteins were expressed in a bacterial expression system. Their ability to induce recall T-cell responses as well as IFN-γ production was evaluated *in vitro* using a lymphocyte proliferation and IFN-γ ELISPOT assay, respectively. The five ORFs presented as a DNA vaccine were further investigated for their ability to induce protective immune responses in sheep against *E. ruminantium* infection following a needle challenge.

All five recombinant proteins were successfully expressed *in vitro* and four were shown to be present mainly as inclusion bodies even though they were also present in the soluble fractions at very low concentrations. Five of the tested proteins were shown to induce T-cell proliferative responses as well as IFN-γ production in PBMC from immune animals. However the responses induced were variable at different test concentrations and also varied amongst the different immune animals.

The corresponding five genes were incorporated into a pCMViUBs vaccine vector and tested as a cocktail in sheep using the DNA prime-protein boost immunization regimen. The cocktail of the five DNA constructs provided 20% protection against a virulent *E. ruminantium* (Welgevonden) needle challenge. Sheep vaccinated with the cocktail DNA vaccine showed increased T-cell proliferative responses and IFN-γ production before challenge. However, this was decreased after challenge in sheep that succumbed to the disease and increased in the sheep that survived challenge. Further testing of the five ORFs as single gene constructs and dose optimization is necessary and may improve the response obtained.

The evaluation of the susceptibility of *Trypanosoma congolense* isolates collected from cattle and buffalo in KwaZulu-Natal to isometamidium chloride and diminazene aceturate

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Early detection of drug resistance in *Trypanosoma* spp. in the field is a prerequisite for adequate implementation of control strategies to limit its spread. Even though trypanocides have been used for years in KwaZulu-Natal, it is still not known whether trypanosomes circulating in livestock have already developed resistance to any of the drugs available in the market.

To answer this question, a total of 11 *Trypanosoma congolense* Savannah-type isolates were assessed for drug resistance to isometamidium chloride (ISM) and diminazene aceturate (DA). Resistance testing was performed in mice using the single-dose protocol. The isolates used were collected from cattle (n=6) and African buffaloes (n=5). For each isolate, three groups of six mice were inoculated intraperitoneally with 10^5 trypanosomes in 0.2 mℓ of a PSG solution. Twenty-four hours later, mice belonging to two groups were treated intraperitoneally with a solution of ISM (1mg/kg) and DA (20mg/kg), respectively. The third group was kept as control. The presence of parasites in the blood was checked twice a week by microscopical examination of a drop of blood collected from the tail. Mice were followed up for relapses up to 60 days post-infection.

Relapses were observed in single mice in four groups, i.e. infected with four different isolates, respectively. One isolate originated from cattle and three from buffalo. One relapse concerned ISM (cattle isolate) and three concerned DA (buffalo isolate). According to the protocol used, an isolate was considered as resistant when at least two of the six mice relapsed. Since only one relapse was observed from each of the four isolates, the 11 isolates tested can all be considered as sensitive to both drugs.

The results of this study suggest either drug resistance is not present in the study area or the prevalence of isolates that are resistant to either DA or ISM is be very low. Even though this result was predictable in isolates collected from buffalo since these animals are not treated, the lack of resistance in isolates collected from cattle requires further investigation using a large number of isolates. If this is confirmed, this result will be an indication that the actual situation of drug resistance in KwaZulu-Natal is not alarming.

An update of the bovine trypanosomiases situation at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal Province, South Africa

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In South Africa, the distribution of tsetse flies has undergone considerable changes over the last century. The rinderpest epidemic of 1896 and intensive aerial spraying operations between 1946 and 1952 have resulted in the eradication of two savannah species, *Glossina morsitans morsitans* and *G. pallidipes*. However, *G. austeni* and *G. brevipalpis* persisted in KwaZulu-Natal Province. Since the nagana outbreak of the 1990s, little information is available on the prevalence of the disease in cattle.

The aim of this study was to obtain updated data on and assess the contribution of trypanosomiases to the disease burden of cattle kept at the edge of the Hluhluwe-iMfolozi Park, KwaZulu-Natal Province, South Africa.

A survey was conducted at Mvutshini diptank adjacent to the northern edge of the tsetse-infested area. A purposeful sampling strategy was used by restricting sampling to animals that the livestock owner considered to be in poor condition. A total number of 76 adult (12 months of age) communal cattle (Angoni breed) were sampled. From each animal, jugular blood was collected in vacutainer tubes with EDTA as anti-coagulant. Molecular polymerize chain reaction (PCR), restriction fragment length polymorphism and parasitological techniques (packed-cell volume (PCV) and wet smear preparation) were used to analyse the samples.

Of a total of 76 blood samples collected, 26 (34.2%) were parasitologically positive and 46 (60.5%) were positive on PCR-RFLP. All parasitologically positive animals were also positive on PCR-RFLP. Almost all infections were due to *Trypanosoma congolense* savannah subgroup. The mean PCV of all animals sampled was 19.8 ± 4.2%. The mean PCV of parasitologically positive animals (18.6 ± 3.8%) differed little ($P>0.05$) from the mean PCV of parasitologically negative animals (20.5 ± 4.4%). Similarly, the mean PCV of animals positive on PCR-RFLP (19.6 ± 4.3%) differed little ($P>0.05$) from the mean PCV of animals negative on PCR-RFLP (20.2 ± 4.3%). A total of 63 animals had a PCV≤ 24%.

This finding suggests that trypanosomiases is still a problem in the study area. However furthers studies are needed to clarify the low PCV values observed in PCR negative animals.

Molecular analysis of *M. bovis* isolated from buffaloes in Hluhluwe-iMfolozi Park, South Africa

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Bovine tuberculosis (BTB), a chronic disease of mammals, is a threat to South African wildlife. The aetiological agent of BTB, *Mycobacterium bovis*, has a wide host range including buffalo which is a reservoir host in South African wildlife populations. This study reports the molecular typing of *M. bovis* isolated from buffalo in Hluhluwe iMfolozi Park (HiP) by utilizing tools such as PCR based analysis of deleted genomic regions, Multiple Locus Variable [number of tandem repeats] Analysis (MLVA) and spoligotyping.

Bronchial lymph node tissue samples (n=12) from buffaloes which previously tested positive to the Single Intradermal Comparative Tuberculin Test (SICTT) were cultured using standard methods. DNA for PCR was obtained by heating a loop from pure culture colonies at 80 °C for 1 hour. Deletion analysis, amplifying the regions of difference (RD); RD4 and RD9 were done on the isolates. Also PCR, amplifying 16 variable number of tandem repeats (VNTR) loci using previously described conditions and spoligotyping analysis were carried out on the samples.

Four of the 12 samples were positive after mycobacterial culure. The deletion of both RD 4 and RD 9 confirmed the isolates as *M. bovis*. After MLVA genotyping, 2 distinctly different VNTR profiles were observed. MLVA analysis based on ETR A-E highlighted variations at 4 of the 5 ETR-loci, as well as at 2 other loci (QUB 11A and MTUB 12). Similarly, two different spoligopatterns belonging to two different families were observed. The results obtained by spoligotyping revealed 2 different spoligopatterns. A unique spoligopattern common to 3 isolates was identified and marked by the absence of spacers 3, 6, 8-12, 16, 22 and 23, as well as spacers 39-43 in the direct repeat region, which is typical of *M. bovis* isolates. This spoligopattern is not described in *M. bovis* as well as the International spoligotype database, Spol Db4. On the basis of its unique pattern and origin, this strain was given the nomenclature BOVIS2_HiP and subsequently included on the SpolDB4 database. The spoligopattern of the 4th strain, is also not described in the in the *M. bovis* database, but according to the SpolDB4 database nomenclature, this strain belongs to the family 481 BOVIS1.

This study showed that at least three *M. bovis* strains could be characterized in HiP, when compared with previous characterization of *M. bovis* in HiP where one strain was earlier identified, thus indicating that there has been more than one introduction of BTB in this area. The study emphasizes the need for a more thorough molecular study in order to assess the origin of strains and the routes of transmission between different animal species.

Genetic characterization of dog rabies viruses from Nigeria

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Rabies is a zoonotic disease endemic in Nigeria similar to other parts of Africa. The disease is caused by highly neutrotropic viruses belonging to the *Lyssavirus* genus and not only poses a serious public health risk, but considerable economic burden through losses to livestock and the high cost of post-exposure management. The true incidence and prevalence of the disease is largely unknown due to the current surveillance system which is characterised by misdiagnosis and gross underreporting. A study was therefore undertaken to elucidate the molecular epidemiology of dog rabies through establishing the phylogenetic relationships of dog rabies viruses in Nigeria.

One hundred available rabies viruses all recovered from the domestic dog between 1989 and 2008 were included in this investigation. A tenth (10%) of the samples were confirmed to contain lyssavirus antigen by the fluorescent antibody test (FAT). Total viral RNA extractions were performed on original brain tissues using TRI reagent (Sigma, Aldrich, USA) as described previously. Reverse transcription (RT) was performed using approximately 1 µg of total viral RNA and partial regions of glycol-protein and nucleoprotein genes were targeted for amplification using the (G+/L- and Lys001+/550B-) primer sets respectively. The PCR amplicons were purified using the Wizard® SV Gel purification system (Promega, USA) and cycle sequenced using the Big Dye Terminator V3.0 sequencing kit (Applied Biosystem) with the same primers as in the amplification steps. Electrophoretic analysis of the sequencing products was carried out on an automated Applied Biosystem 377 DNA sequencer. Multiple nucleotide sequences were generated using Clustal W and statistically evaluated using the bootstrap resampling technique. Graphic outputs were visualized using the TREEVIEW program.

The investigation revealed that all the dog rabies viruses analysed in the study sample are very closely related with a mean sequence homology of 93.2%. However, despite this close genetic relationships between the viruses, 3 distinct groups (designated A, B and C) with high significant statistical support values were delineated. The major group (A) was composed of virus isolates from the North Central region of the country and comprised 4 sub-groups possibly reflecting localized rabies outbreaks. The other two groups were viruses originating from the North East and South West regions of Nigeria.

These data suggest that there is a single dominant lineage of rabies circulating in this host species and that parenteral vaccination of this principal vector species remains the primary method for the control of the disease in this West African country.

Identification of a small *Babesia* species found in a dog imported from Taiwan

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Canine *babesiosis* is caused by tick-transmitted intraerythrocytic protozoan parasites occurring worldwide. These were previously classified according to their morphological appearance as *Babesia canis,* the "large" species and *Babesia gibsoni,* the smaller species. Recently, molecular studies have shown three vector-specific subtypes of *Babesia canis: B. c. rossi* found in South Africa, *B. c. vogeli* found worldwide, and *B. c. canis* found in Europe. It has also been shown that the "small" *Babesia* parasites consist of three morphologically similar but genotypically distinct parasites: *Babesia gibsoni,* Asia type (found in north and eastern Africa and North America); "*Theileria*" *annae*, a *Babesia* parasite of dogs in Spain; and *Babesia conradae* identified in dogs in California.

In South Africa, imported dogs are required to be tested for *Babesia gibsoni* by means of examination of thin Giemsa-stained blood smears for the presence of intraerythrocytic trophozoites and the Indirect Fluorescent Antibody (IFA) test for the presence of antibodies in the serum. Recently, a blood sample from an 8-year-old Border Collie cross being imported from Taiwan was sent to be tested for *Babesia gibsoni* according to protocol. The sample tested positive on the IFA test at a reciprocal titre of 1:80. The trophozoites that were observed on the Giemsa-stained thin blood smear were "small". For further confirmation of the small parasite being *Babesia gibsoni,* DNA was extracted from 200 µl of EDTA blood. The V4 variable region of the 18S rRNA gene were amplified and subjected to the reverse line blot (RLB) hybridization assay as previously described. PCR amplicons did not hybridize with the *Babesia gibsoni*-specific probe as expected. It hybridized only with the *Babesia* genus-specific probe, which could suggest the presence of a novel species or variant of a species.

Since the RLB membrane did not contain probes specific for *T. annae* or *B. conradae*, the presence of these species could not be excluded. To elucidate the identity of the parasite present, the full length 18S rRNA gene will be amplified, cloned and sequenceed. Sequence data will be analyzed package and aligned with published sequences of related genera.

Determination of the minimum protective dose for bluetongue serotype 2, 4 and 8 vaccines in sheep

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Bluetongue (BT) live attenuated virus vaccine has been used successfully in the control of BT in southern Africa and Europe. However, concerns about the safety, possible development of viraemia and clinical signs post vaccination (PV) presented an opportunity to investigate the possibility of reducing the current BT vaccine titre to below 10^4 PFU/ml.

A total of 83 merino sheep were used and vaccinated with BTV monovalent vaccine containing serotypes 2, 4 and 8 with the following titres: 10^2, 10^3 and 10^4 PFU/mℓ. Positive and negative controls for each serotype were used. Animals were bled on day 0, 3, 6, 9, 12, 15, 18, 21, 25 and 28 PV and tested for viraemia. Seroconversion was tested PV on day 0, 3, 9, 15, 21, 6 weeks, 3 and 4 months. Vaccinated sheep were then challenged at 6 weeks using BTV-infected blood and at 4 months using cell cultured material and evaluated for 14 days for clinical reaction index.

Seroconversion was demonstrated in some of the sheep vaccinated at a low titre of 10^2 PFU/ml from day 9 PV. Sheep vaccinated with serotype 8 did not demonstrate any form of viraemia both PV and post challenged at all titres. However, sheep challenged with serotype 4 developed mild clinical signs for less than 3 days. Sheep challenged with cell cultured BTV serotype 2 also showed mild clinical signs and also developed viraemia that lasted for less than 3 days which was also demonstrated in sheep vaccinated with a titre of 10^4 PFU/mℓ.

It was clearly shown that BTV monovalent vaccine containing serotypes 2, 4 and 8 with titres below 10^4 PFU/mℓ can protect more than 90% of vaccinated animals against clinical disease. Although certain serotypes failed to protect against infection, all serotypes protected against the development of clinical disease when challenged with both BTV-infected blood and cell cultured material.

Detection of feline coronavirus in cheetahs using real-time PCR

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Feline coronavirus is a highly contagious virus and can be a serious pathogen of members of the family *Felidae*. It is transmitted via the gastrointestinal and respiratory tracts. The genome of the virus is 27-31 kilobase pairs long and it is believed that the 7b ORF gene plays a role in the virulence of the virus and that the 7a ORF plays a role in the development of disease. Genes 7a and 7b were used as target genes in the realtime PCR assay to study the prevalence of coronavirus in captive populations of cheetahs[1,2].

Faecal specimens were collected from healthy cheetahs from different conservation centres and samples were also obtained during necropsy from cheetahs after various causes of death. RNA was extracted using the QIAamp® Viral RNA mini kit (Qiagen, Southern Cross Biotechnologies) as protocol. A one-step RT-PCR using Superscript III Platinum One-Step qRT_PCR System was performed using the manufacturer's recommendations. Primers to a conserved region of the 7b gene of coronavirus as well as a probe (TaqMan, Applied Biosystems) specific to an internal genetic region of the target were used[3].

Only 0.68% of the samples tested positive but some of those yielded late peaks. One of the latter samples was collected from a clinically sick cheetah called "Jake". The other samples with late peaks were collected from animals that did not have clinical disease.

Late peaks require further investigation, particularly the sample collected from the sick cheetah. The low number of positive samples may be due to low levels of viral shedding or the fact that the probe used is not specific enough. A new probe must be designed that targets a bigger area in the gene of interest. More sequencing results are therefore required.

References

1. Everman J F 1986 Feline coronavirus infection of cheetahs. *Feline Practice.* 16: 21-30.
2. Hoskin J D 1995 Perspective on feline coronavirus evolution. *Feline Practice.* 23:34-39.
3. Kennedy M A, Dolorico T, Hillis, McNabb A, Serino Moffat A, Kania S 2006 Genetic mutations of the 7a7b genes of coronavirus of cheetahs (*Acinonyx jubatus*). *American Journal of Veterinary Research* 67:627-32.

Serum biochemistry changes in virulent canine babesiosis

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Virulent canine babesiosis is characterised by marked and life threatening changes in homeostasis. These changes are partly reflected in the serum biochemistry parameters of patients suffering from severe and complicated canine babesiosis. Various smaller studies have briefly investigated the serum biochemical and other electrolyte changes, but failed to ascertain and publish their association with mortality in canine babesiosis molecularly confirmed to be caused by *Babesia rossi*.

A prospective study was undertaken to determine the serum biochemistry concentrations of dogs with canine babesiosis at presentation and observed the associated mortality. Ninety five patients were studied. The initial diagnosis of canine babesiosis was made on stained thin capillary blood smears. Diagnosis and babesia subtype was confirmed as *B. rossi* and all patients were negative for *Ehrlichia canis* by polymerase chain reaction (PCR) and reverse line blotting (RLB). Three outcomes were defined: dogs treated as outpatients, (n = 32); hospitalised dogs that survived, (n = 56); and hospitalised dogs that died, (n = 7). The following biochemical parameters were determined: total protein, albumin, globulin, creatine kinase (CK), creatinine, alkaline phosphatase (Alp), alanine aminotransferase (Alt), bile acids, glucose, sodium (Na), potassium (K), chloride (Cl), ionised calcium (Ca), magnesium (Mg), phosphorus (P), amylase and lipase. Data was tested for normality with the Kolmogorov Smirnov test and analysed by the non-parametric Kruskal Wallis test for comparison of more than two groups. To compensate for the testing of multiple parameters, significance was set at $P < 0.001$. Data is expressed as the median value.

Overall mortality was 7/95 (7.5 %). Median serum bile acid, Mg and P concentrations were significantly higher ($P < 0.001$) while median Ca concentrations were significantly lower ($P < 0.001$) in dogs that died. Less severely affected dogs treated as outpatients had significantly lower median serum CK ($p < 0.001$) concentrations than the other two groups. No significant differences at $P < 0.001$ were detected between the groups in any of the other parameters.

This study demonstrated that mortality was associated with elevated bile acid, Mg and P and with lower ionised Ca concentrations in dogs with virulent *B.rossi* infection. Lower serum CK concentrations were clearly associated with milder disease, while elevated bile acid concentrations support the notion of hepatic dysfunction playing a role in adverse outcome. The respective roles of elevated serum Mg and P and decreased calcium concentrations warrant further study.

Admission serum biochemical parameters are not associated with mortality in puppies with parvo viral diarrhoea

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Parvoviral diarrhoea is a disease process culminating in sepsis. Elevated serum cortisol and decreased thyroxine and white blood cell concentrations have been shown to predict mortality in parvoviral diarrhoea. However, the association of serum electrolytes and other biochemical parameters with mortality in puppies with parvoviral diarrhoea have been poorly described.

A prospective, case controled study was undertaken to determine the admission serum electrolyte and other biochemical concentrations of puppies admitted to a high care isolation ward with severe parvoviral diarrhoea and observed the associated mortality. Sixty three patients and 17 control puppies were studied. The diagnosis of parvoviral diarrhoea was confirmed by the detection of viral particles by faecal electron microscopy. Blood samples were taken prior to treatment. Serum albumin, sodium, potassium, ionized calcium, magnesium, urea and creatinine concentrations were compared between puppies with parvoviral diarrhoea and control puppies and between survivors (n=50) and non-survivors (n=13) using the Mann Whitney U for non-parametric data.

Overall mortality was 21% (13/63). Median age and bodyweight was 4 months and 5 kg respectively. Median duration of illness prior to admission was 3 days. Median serum Na, K, Ca, Creatinine ($P < 0.001$) and Urea concentrations ($P < 0.05$) were significantly lower, whereas median serum magnesium concentration was significantly higher ($P < 0.05$) in parvoviral diarrhoea puppies compared to control dogs. Serum albumin concentrations did not differ between cases and controls. Furthermore, there were no differences in the median serum concentrations of any of the measured parameters between survivor and non-survivor group puppies with parvoviral diarrhoea.

Serum biochemistry and electrolyte parameters are markedly affected in dogs with parvoviral diarrhoea. The severe vomiting and diarrhoea were likely responsible for the lower concentrations of sodium and potassium. Ironically, serum urea and creatinine concentrations were lower in puppies with parvo viral diarrhoea than in control puppies, despite the marked dehydration, which was probably caused by the antecedent anorexia and low muscle mass of the parvoviral diarrhoea puppies. Unlike cortisol or thyroxine or white cell count, no individual biochemical parameter was able to predict mortality in puppies with parvoviral diarrhoea.

Prevalence of and risk factors for feline hyperthyroidism in Hong Kong

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Feline hyperthyroidism is an important disorder in middle-aged and old cats. The cause and pathogenesis is still unknown and there are few published incidence rates or prevalence estimates.

A descriptive, cross-sectional study was undertaken to determine the prevalence of and potential risk factors for feline hyperthyroidism in Hong Kong. Serum thyroxine (T_4) concentrations were measured in 305 aged cats that presented at various veterinary clinics in Hong Kong between June 2006 and August 2007. Data was collected about the health of the cats as well as their vaccination history, internal and external parasite control, diet and environment. Serum total T_4 concentration was determined by use of commercially available radioimmunoassay kits (Coat-a-count®, DPC®). For total T_4 the normal reference range used was 12.8 – 50 nmol/L (1.0-3.9ug/dL). All cats with serum total T_4 concentrations more than 50 nmol/L were classified as hyperthyroid. Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured on all the samples. Prevalence of hyperthyroidism with exact binomial 95% confidence intervals was calculated for all cats combined, for cats classified as healthy and for cats classified as sick. Univariable associations between potential risk factors, clinical signs, raised ALT and raised ALP and hyperthyroidism were assessed using a two-tailed Fisher's exact test. A multiple logistic regression model was used to estimate the effect of the risk factors on the development of hyperthyroidism. The fit of the final logistic regression model for risk factors was assessed using the Hosmer-Lemeshow goodness-of-fit test.

The prevalence of hyperthyroidism in Hong Kong was estimated at 3.93% (95% CI: 2.05-6.77) and there was no significant difference in prevalence between healthy (3.16%) and sick (4.37%) cats ($P = 0.76$). There was no clear relationship between sex, vaccination status, parasite control, indoor environment or the consumption of canned food and the development of hyperthyroidism. Domestic shorthair cats were less likely to be diagnosed with hyperthyroidism (OR = 3.34, 95% CI = 0.94-11.86) and the two older age groups of cats were more likely to be diagnosed with hyperthyroidism (OR = 2.77; 95% CI = 0.82-9.4 and 11.88; 95% CI = 1.06-133.7, respectively). There were no characteristic clinical features amongst the cats that had early hyperthyroidism. The presence of the following factors was significantly associated with hyperthyroidism: polyphagia, diarrhea, palpable thyroid nodule and raised ALT and ALP.

This study concluded that the prevalence in Hong Kong is less than in most other parts of the world, despite the presence of previously identified risk factors.

Major outer membrane proteins of *Ehrlichia* species are mostly polymorphic and have evolved by gene duplication and genetic drift without positive selection pressure

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Ehrlichia species have families of paralogous genes which code for major outer membrane proteins (omps), many of which are both polymorphic and immunogenic. Different analysis tools were used to develop insights into the evolution and possible function of these families.

The sequences of omp gene families from four *Ehrlichia* species were examined. Comparisons were visualised using Artemis Comparison Tool (ACT), phylogenies were inferred using a maximum likelihood algorithm (PHYML), and selection pressure was estimated using Phylogenetic Analysis by Maximum Likelihood (PAML).

The omp genes are of similar lengths, have similar structural features, occur mostly in tandem groups, and have pairwise amino acid identities which are well above the 25% identity limit for a common origin. *E. ruminantium map*1 gene, a single copy in its genome, has multiple orthologs in the other genomes. One of the paralogs, p28-14 (*E. chaffeensis*), p30-10 (*E. canis*) and *map*1-1 (*E. ruminantium*), contrasts with the others in being more conserved and in being expressed solely in the tick vector. Synonymous/non-synonymous nucleotide substitution rates were estimated for *map*1 and at the 95% confidence level one substitution model predicts that one codon may be under weak positive selection. Two other models predict no positive selection for any codon.

The omp gene families appear to have evolved by gene duplication and are changing relatively rapidly. Duplication of an ancestral *map*1 gene has resulted in the different genomes having different numbers of copies of this ortholog. Since the *map*1 gene is not under selection pressure it must owe its polymorphism to accumulation of neutral mutations, which may also explain the relatively rapid rate of change in the gene families as a whole. The exception is *map*1-1 and its orthologs, which is uniquely expressed in the tick vector and probably has a vital role to play in establishing infection in the mammalian host. The immunodominant MAP1 (and orthologs) may act to screen the more conserved MAP1-1 proteins from immune recognition.

STR analysis: isolating DNA from semen smears on microscope slides

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For many commercially important animal species, semen is often analyzed microscopically to study sperm morphology and investigate problems related to fertility in male animals. DNA typing is used routinely for individual animal identification and parentage verification. In order to confirm the identity of the animal from which a specific slide mounted semen sample was derived a technique to obtain the DNA profile from such samples was required.

Microscope slides containing semen smears from stallions and dogs were obtained from the Reproduction section of the Department of Production Animal Studies of the University of Pretoria. Samples had all been exposed to various treatments that might inhibit DNA amplification. Oil was wiped from the surface of the slides with a paper towel. Cover-slips were removed by separation from the slide with a scalpel blade after soaking in an acid/ethanol mixture. Approximately 2μℓ semen was recovered per slide and used in a standard PCIA (Phenyl-Chloroform Isoamylalcohol) extraction protocol with the addition of Dithiothreitol (DTT).

Eosin/Nigrosin stain present on certain slides inhibits PCR and interferes with the accurate quantification of DNA after extraction. Stains were removed with a 37% HCl and 70% ethanol solution which proved effective enough to remove residual stain but mild enough not to influence DNA yield. Slides from which the stain had been removed were found to amplify as efficiently as unstained samples during PCR using standard multiplex PCR. Slides containing a cover slip or oil produced the lowest yield of DNA after extraction and this did not amplify. A similar scenario was seen in the case of samples containing microscope oil on the surface. The samples from the dog demonstrated that the DNA yield from fresh, unstained semen applied to a slide is comparable to that of DNA extracted from blood samples. A freeze-thaw cycle before application of the semen to the slide reduced the DNA yield but amplification was not affected.

It is therefore feasible to use semen smears stored on microscope slides as a source of DNA for individual identification of an animal or to relate parentage results of a mating to an individual sire's previously stored semen characteristics. Stained smears can also be used, provided the stain is removed appropriately.

Sample	Date Collected	Treatment	DNA Concentration (ng/μl) (20-120*)	A260/280 (1.7-1.9)*
Stallion 1	14/11/07	Stain not removed	231.37	0.54
Stallion 1	14/11/07	Unstained	89.86	1.42
Stallion 2	25/01/02	Stain removed	49.73	1.26
Stallion 3	07/03/06	Microscope oil on surface	19.56	1.14
Stallion 4	02/10/07	Cover slip and fixative	0.25	0.05
Dog 1	27/03/00	Blood sample for comparison	274.67	1.76
Dog 1	27/03/08	Unstained	209.55	1.42
Dog 1	27/03/07	Freeze-thawed. Unstained	68.68	1.48

* Ideal

Membrane specialisations in the efferent ducts of the ostrich epididymis

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The epididymis (epididymal region) of birds is a mass of tissue located on the latero-medial aspect of the testis, is continuous with the defferent duct, and contains all the elements (rete testis, efferent ducts, connecting ducts and ductus epididymis) of the excurrent duct system. Although the structure of the efferent ducts of the ostrich has been described in some detail, little information is available on the location and characteristics of membrane specialisations. This study provides comparative morphological data on the relationship and mode of contact between the epithelial cells forming the lining of the proximal and distal efferent ducts in the ostrich.

Specimens of the epididymis were obtained from three sexually mature birds slaughtered at the Oudtshoorn abattoir. Tissue blocks were immersion-fixed in 4% glutaraldehyde in Millonig's phosphate buffer, post-fixed in similarly buffered osmium tetroxide and routinely prepared for transmission electron microscopy.

The most prominent membrane specialisation observed was the apicolateral junctional complex which was composed of three distinct regions: (a) focal tight junctions (*zonula occludens*) beneath the lumen. (b) short stretch of closely apposed membranes enclosing a narrowed intercellular space. (c) A well developed adhering junction (*zonula adherens*).

The apicolateral junctional complex appeared similar in both the proximal and distal components of the efferent ducts and occurred between both cell types (ciliated and non-ciliated cells) making up the epithelial lining of the ducts.

In contrast, the lateral cell membranes differed markedly between the two ducts. The proximal duct displayed long stretches of complex cytoplasmic interdigitations and enlarged intercellular spaces whereas in the distal duct adjacent cells simply lied alongside each other forming a consistently regular intercellular space. Adjacent cells in both ducts were linked by desmosome-like structures, that resembled weakly developed adhering junctions (*zonula adherens*). In both ducts the basal plasmalemma of all cells was attached to the underlying basal lamina by an extensive network of hemidesmosomes).

This study revealed a general similarity in the membrane specialisations between the proximal and distal components of the efferent ducts of the ostrich. However, the complexity of the lateral cell membranes of the proximal duct would seem to further support the observation (based on additional morphological features) that the lining cells of this region possess an efficient fluid-absorption capability in contrast to those of the distal efferent duct. It is also not clear why the desmosome-like junctions linking the lateral membranes are relatively poorly developed in comparison to true desmosomes which generally link the lateral cell membranes of adjacent epithelial cells.

Admixture and founder origins in captive cheetahs (*Acinonyx jubatus*) detected using spatial Bayesian clustering

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South Africa has over 500 captive cheetahs (*Acinonyx jubatus*) housed in 44 facilities. Of the cheetahs imported into South Africa for breeding within the past 26 years, an estimated 70% were of Namibian ancestry, leading to valid concerns on the lack of founder diversity in the captive gene pool. Understanding founder contributions are critical for maximising diversity within extant and newly established cheetah populations. The population at the De Wildt Cheetah Breeding Station and Research Centre (DWCBSRC) near Pretoria, the largest contributor of captive cheetahs worldwide, was investigated for its Namibian ancestry.

DNA was extracted from whole blood, tissue and serum from the DWCBSRC captive group (n = 152), free-ranging Namibian cheetahs (n = 51) and southern African wild cheetahs (n = 71) from the Kalahari area in Botswana, and Kuruman, Tosca, Bray, Verglee, Madikwe, Dwaalboom, Thabazimbi, Ellisras, Potgietersrus, Bela-Bela, Messina, Alldays, and Phalaborwa in South Africa. GPS data, when available, was recorded at the point of capture. Thirteen domestic cat (*Felis catus*) microsatellites were multiplexed, PCR products sequenced and fragments separated on an ABI 3130 XL DNA analyzer, and results captured using *STRand*. Standard amplification, typing quality, consistency checks and null allele estimations were concurrently done.

Admixture analysis was done using Structure *2.2*, and after verification and addition of spatial data, spatial Bayesian clustering was completed using *TESS* 1.1. Cryptic structures and ancestry were then detected using *BAPS 3.2*. DWCBSRC cheetahs distinctly cluster into groups, with the *King* phenotype lineage forming 37% of the total captive group. An admixed cohort, with most founders from the former Eastern Transvaal, comprised 27% of the total. The North West and Northern Province have an ancestry representation of 16 and 7%, respectively. Namibian genetic introgression was detectable only in 13% of DWCBSRC cheetahs.

The genetic admixture results from a representative South African captive cheetah population indicate significantly lower Namibian genetic introgression than previously thought. Bayesian clustering tools that can be trained to assign unknown cheetahs to genetic clusters based on samples with known spatial data, has allowed us to revise some of the assumed and unknown ancestral origins of captive-born cheetahs. Current and active re-introduction programmes will benefit in knowing the genetic antecedents of a rescued/trapped cheetah, for legal, forensic and conservation purposes.

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