Doctoral Celebration Programme

Lecture hall 1-35
Arnold Theiler Building

Wednesday, 3 April 2019

Make today matter
Programme

08:55 – 09:00 Welcome by the Dean, Prof Vinny Naidoo

09:00 – 09:20 Prof Martin Schulman introduces Margaret Bethaline Nolan
Efficacy and safety of recombinant zona pellucida vaccines in domestic horse mares and current application of native porcine zona pellucida vaccines in African elephant cows

09:20 – 09:40 Prof Lyndy McGaw introduces Ibukun Michael Famuyide
Potential of Eugenia and Syzygium species (Myrtaceae) to combat diarrhoeagenic pathogens with emphasis on porcine enterotoxigenic Escherichia coli

09:40 – 10:00 Prof Janusz Paweska introduces Gaby Ermelindo Roberto Monteiro
Mutation of adjacent cysteine residues in the NSs protein of Rift Valley fever virus results in loss of virulence in a murine model infection

10:00 – 10:20 Dr Otto Koekemoer introduces Harry Gay Ngoveni
Characterizing the effect of mutation, recombination and reassortment on the genetic diversity of African horse sickness virus genomes

10:20 – 10:40 Coffee/tea with snacks in foyer

10:40 – 11:20 Official photos

11:20 – 11:40 Prof Henriette van Heerden introduces Kgagelo Edward Lekota
Genomic study of Bacillus anthracis and Bacillus species isolated from anthrax outbreaks in South Africa

11:40 – 12:00 Prof Christo Botha introduces Hamza Ibrahim Isa
Development and evaluation of immunogens for a yellow tulp (Moraea pallida) vaccine

12:00 – 12:20 Prof Henriette van Heerden introduces Nurudeen Olalekan Oloso
Prevalence and characterization of Salmonella isolates originating from the broiler production value chain in Nigeria

12:20 – 12:40 Announcement by the Dean, Prof Vinny Naidoo
Refreshments
In her thesis, Efficacy and safety of recombinant zona pellucida vaccines in domestic horse mares and current application of native porcine zona pellucida vaccines in African elephant cows, the promovenda reported that a recombinant zona pellucida (reZP) immunocontraceptive vaccine formulated with novel adjuvants was an effective and safe alternative to porcine zona pellucida (pZP) vaccination in mares. Data collected in randomised controlled studies described mares’ ovarian effects, antibody titre responses and safety profiles. Additionally, a retrospective data analysis from elephant populations in South African reserves demonstrated pZP immunocontraceptive vaccination as a successful management tool, informing future implementation. Overall, this work reported novel and improved alternatives for humane veterinary population management of horses, potentially similarly applicable in elephants and other species.
In his thesis, Potential of Eugenia and Syzygium species (Myrtaceae) to combat diarrhoeagenic pathogens with emphasis on porcine enterotoxigenic Escherichia coli, the promovendus worked towards providing solutions to the one health issue of antimicrobial resistance via the food chain by investigating replacements for synthetic antibiotics commonly added to livestock feed in the form of medicinal plant extracts. **In vitro** methods were used to determine the antimicrobial, anti-inflammatory and antioxidant activities, cellular safety and mode of antibacterial action of nine South African Eugenia and Syzygium medicinal plant species. Plant extracts had noteworthy antibacterial activity against planktonic and biofilm forms of various Gram-positive and Gram-negative bacteria as well as good anti-quorum sensing and anti-adhesion activities. The plants had high antioxidant and anti-inflammatory activities and low cytotoxicity to mammalian cells. The results provide support for the potential development of some of the selected plants as phytogenic feed additives.

**Supervisor**: Prof LJ McGaw  
**Co-supervisor**: Prof JN Eloff  
**External examiner**: Dr AO Aremu (North-West University)  
**External examiner**: Dr M Walkenhorst (Research Institute of Organic Agriculture, Switzerland)

In her thesis on “Mutation of adjacent cysteine residues in the NSs protein of Rift Valley fever virus results in loss of virulence in a murine model infection”, the promovenda aimed to investigate the effects of non-structural protein (NSs) conserved cysteine substitutions on RVFV virulence. RVFV is a mosquito borne zoonotic RNA virus which has a high potential to cause large outbreaks in livestock and humans, resulting in severe health and socio-economic losses. No human vaccines are commercially available. The S segment encodes a NSs, the major virulence factor. NSs gene has five highly conserved cysteine residues at positions 39, 40, 149, 178 and 194. The mutation at cysteines 39 and 40 (C39S/C40S) resulted in attenuated phenotype in BALB/c mice. This study provides a better understanding of RVFV molecular mechanisms governing its virulence and indicates that RVFV attenuated mutant (C39S/C40S) can be used as a safe virus for serum neutralization based assays as well as a potential vaccine candidate.

**Supervisor**: Prof JT Paweska  
**Co-supervisor**: Dr P Jansen van Vuren  
**External co-supervisor**: Dr J Kortekaas (Utrecht University, The Netherlands)  
**Internal examiner**: Prof W Markotter  
**External examiner**: Prof FJ Burt (University of the Free State)  
**External examiner**: Prof S Günther (Bernhard-Nocht-Institute for Tropical Medicine, Germany)
In his thesis, Characterizing the effect of mutation, recombination and reassortment on the genetic diversity of African horse sickness virus genomes, the promovendus made an in depth study of the evolution in the genome of the African horse sickness virus through the application of massively parallel sequencing of the complete genomes of close to 100 viruses, isolated over a period of more than 60 years. It was determined that genetic mutations are under strong purifying selection and that the mean nucleotide substitution rate is lower than the case for other RNA viruses, a constraint imposed by the necessity to replicate in both the host and insect vector species. Widespread reassortment of genome segments was detected and it was shown for the first time that recombination events occurred within some genome segment. This knowledge will be used in the development of the next generation of vaccines to ensure complete antigenic matching between vaccines and field viruses.

Anthrax is a zoonotic disease that naturally occurs in wild and domestic animals, caused by Bacillus anthracis. Bacillus anthracis and B. endophyticus isolated from anthrax cases in the Northern Cape Province (NCP) had similar microbiological and genetic features. Whole genome sequencing identified the homologous polyglutamate genes present in both species and also identified regions that differentiated B. anthracis from B. endophyticus. Molecular analysis of the Kruger National Park (KNP) and NCP strains from anthrax outbreaks in animals clustered mostly in the A-clade. The rare B-clade strains were last isolated in the 1990s in KNP. In this study, Genomic study of Bacillus anthracis and Bacillus species isolated from anthrax outbreaks in South Africa, a B-clade strain was isolated from Limpopo province. A Bacillus anthracis genomic database was generated to identify single nucleotide polymorphisms across sequenced and world-wide genomes. Specific novel SNPs were developed into high-resolution melting SNP discriminative assays. This study confirmed the diversity of B. anthracis strains and identified novel sub-clades and branches in South Africa.

Supervisor : Dr JJO Koekemoer
External co-supervisor : Dr A van Schalkwyk (ARC - Onderstepoort Veterinary Institute)
Internal examiner : Dr WC Fick
External examiner : Dr CDJ Labuschagne (Inqaba Biotechnical Industries (Pty)

Supervisor : Prof H van Heerden
External co-supervisor : Dr CA Hefer (ARC - Onderstepoort Veterinary Institute)
External co-supervisor : Dr E Madoroba (ARC - Onderstepoort Veterinary Institute)
External examiner : Prof AR Hoffmaster (Centre for Disease Control and Prevention, USA)
External examiner : Prof L Kenefic (University of Maryland, USA)
External examiner : Dr S Derzelle (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, France)
In his thesis, Development and evaluation of immunogens for a yellow tulp (Moraea pallida) vaccine, the promovendus investigated if a yellow tulp vaccine could be developed to prevent this economically important plant poisoning in livestock. Epoxyscilliroside, proscillaridin and bufalin were conjugated to different proteins to render them immunogenic. A suitable adjuvant was added before vaccinating rabbits in three different trials. An ELISA was developed to determine antibody response. Antibodies were raised and the antibodies against proscillaridin and bufalin cross-reacted with epoxyscilliroside. The animal vaccination studies were scaled up and sheep were immunized. Cytotoxic effects of epoxyscilliroside were evaluated in a rat embryonic cardiomyocyte cell line using cell viability assays and transmission electron microscopy. The cytotoxicity studies demonstrated that epoxyscilliroside causes cell necrosis. Subsequently, the antibodies raised in sheep were concentrated and purified before evaluation of their in vitro neutralization efficacy. However, the anti-epoxyscilliroside antibodies failed to prevent cytotoxic effects induced by epoxyscilliroside.

In his thesis, Prevalence and characterization of Salmonella isolates originating from the broiler production value chain in Nigeria, the promovendus investigated the epidemiology and established baseline data in support of the prevalence of Salmonella in the Nigeria broiler production value chain (NBPVC) using South Africa as a reference. The structure of the NBPVC revealed policy inconsistency, uncompetitive pricing and compromised food-safety standards. Biosecurity compliance in broiler-breeder-farms was poor with demonstrable high risk of vertical transmission of salmonellosis. Prevalence of 55% for locations and 23% for samples was observed for multidrug resistant Salmonella linked to unregulated high level of drug usage in animal-human-environments interface. The study demonstrated Salmonella as a neglected zoonotic foodborne pathogen in Africa. Joint continental wise surveillance and national data collection platforms on Salmonella with antimicrobial resistance is imperative to establish good antimicrobial stewardship. 

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**Supervisor**
- Prof CJ Botha

**Co-supervisor**
- Dr JE Crafford

**Internal examiner**
- Prof JG Myburgh

**External examiner**
- Prof FN Toka (Ross University School of Veterinary Medicine, West Indies)

**Supervisor**
- Prof FO Fasina

**Co-supervisor**
- Prof H van Heerden

**Internal examiner**
- Prof CME McCrindle

**External examiner**
- Prof L Mughini-Gras (RIVM, The Netherlands)

**External examiner**
- Dr ES Swai (Department of Veterinary Services, Tanzania)
In her thesis, Susceptibility of *Salmonella* serotypes to plant secondary metabolites with application in infection control, the promovenda evaluated the antibacterial activity of leaves of ten selected South African medicinal plants. *Loxostylis alata* was selected for further investigation based on promising preliminary antibacterial and antioxidant activity and low cytotoxicity. The activity of crude extracts of *L. alata* was evaluated against both susceptible and multi-resistant *Salmonella* isolates of animal origin in comparison to commercial antibiotics with good results. No genetic relatedness between resistant strains was observed based on pulsed field gel electrophoresis (PFGE) analysis of *S. Enteritidis* and *S. Typhimurium* isolates. The antibacterial compounds delicaflavone, 5-demethyl sinensetin, methyl gallate and cetene were purified and identified from the leaves of *L. alata* for the first time. The modes of action via antibiofilm activity and immune modulatory properties of *Loxostylis alata* leaf extracts and purified compounds were reported for the first time.

Supervisor: Prof LJ McGaw
Co-supervisor: Prof MM Ehlers-van der Zel
External examiner: Prof V Kuete (University of Dschang, Cameroon)
External examiner: Prof J van Staden (University of KwaZulu-Natal)

In his thesis, Anti-inflammatory and antioxidant activities of selected southern African medicinal plants with potential application in treating helminth infections, the promovendus investigated the biological activities of eleven southern African plants used in traditional medicine to treat inflammation. Two new compounds with good anti-inflammatory and anthelmintic activities were isolated for the first time from *Typha capensis* (isorhamnetin-3-O-β-D-glucoside, and isorhamnetin 3-O-rutinoside). *Typha capensis* had good activity not only against the larvae of the parasitic livestock nematode *Haemonchus contortus* but also against various inflammatory mediators. This supports the potential of *T. capensis*, the common bulrush, for development into an anthelmintic remedy able to additionally enhance healing of damaged tissues via anti-inflammatory and antioxidant action. There was a strong correlation between *in vitro* antioxidant and anthelmintic activities, suggesting that antioxidant assays may be used in bioassay-guided fractionation for isolation of compounds from plants with activity against helminth parasites.

Supervisor: Prof LJ McGaw
External co-supervisor: Dr E Mfotie Njoya (University of Yaoundé, Cameroon)
Internal examiner: Prof N Lail
External examiner: Prof J Hohmann (University of Szeged, Hungary)
External examiner: Dr AR Ndhlala (Agricultural Research Council)
In her thesis, Development and evaluation of an inactivated multi-clostridial vaccine for captive bred southern white rhinoceroses (Ceratotherium simum simum), the promovenda developed a multi-clostridial vaccine using modern single-use technology of C. perfringens type A, C. sordelli, C. novyi, C. septicum and C. chauvoei. Fermentation was followed by the development of indirect enzyme-linked immunosorbent assays (IELSAs) for antibody detection. Two groups of white rhinoceroses were evaluated consisting of a group vaccinated with a commercial multi-clostridial vaccine for cattle and a group vaccinated with Rhinovax. Post-vaccination antibody titres from Rhinovax vaccinated rhinoceros increased significantly (P ≥ 0.05) except against C. perfringens type A when compared to animals vaccinated with the commercial cattle vaccine. To establish baseline data, sero-surveillance of unvaccinated rhinoceros (n=100) in the Kruger National Park was evaluated. Data showed high titres to C. perfringens type A and C. septicum only. The results contributed to the understanding of serological profiles of rhinoceroses to veterinary important clostridial organisms.

Brucellosis is a challenging, zoonotic disease posing a devastating impact on livestock. A novel vaccination strategy employed the avirulent, Escherichia coli and Yarrowia lipolytica expression systems for the surface display of Brucella antigens, Omp16 and Omp19. In her thesis Cell surface display as a potential Brucella antigen delivery system, the promovenda investigated whether the incorporation of these homologous lipoproteins in non-pathogenic cells could ultimately protect livestock against common Brucella species infection. Super resolution localization microscopy confirmed lipoprotein surface distribution, which validated these platforms for use as vaccine "vehicles". This provided insight into Brucella lipoprotein spatial distribution and biogenesis. The vaccine efficacy monitored in mice indicated the humoral immune response acquired was supported by a cross-reactive response to the bacterial envelope components. The protection conferred in the immunized model by the avirulent delivery systems, upon Brucella infection was inadequate in comparison to the B. abortus S19 vaccine. Nonetheless, these antigen platforms coupled with these lipoproteins may demonstrate potential as booster vaccinations.

Supervisor : Prof H van Heerden
Co-supervisor : Dr JE Crafford
External examiner : Prof F Uzal (University California, Davis, USA)
External examiner : Dr II Hitzeroth (University of Cape Town)
External examiner : Dr MJ Miller (Stellenbosch University)

Supervisor : Prof H van Heerden
Co-supervisor : Dr MC Crampton
External co-supervisor : Dr R Roth (CSIR)
Internal examiner : Prof J Theron
External examiner : Dr G Briones (National University of San Martin, Argentina)
External examiner : Dr D O’Callaghan (University of Montpellier, France)
Trypanosomosis, a disease transmitted biologically by tsetse flies and mechanically by tabanids, together with trypanocidal drug resistance, has caused a drastic reduction in the cattle population in Zambezia province. In this thesis, Epidemiology of drug resistance and evaluation of possible mechanical transmission of Trypanosoma congolense by haematophagous insects in Zambezia Province, Mozambique, drug resistance was assessed through a block treatment experiment. An entomological survey to study the composition of the flies involved in the transmission of trypanosomosis, including the determination of trypanosome infection rates, was also conducted. The study revealed the presence of single and multi-drug resistance in Nicoadala district. It was also demonstrated that there is a small tsetse population surviving in the area as well as a large homogeneous population of tabanids with an overlapping distribution, both contributing to the transmission of trypanosomosis. This information is fundamental when considering the control of trypanosomosis in the area.

In her thesis, Genomic comparison of Mycoplasma species isolated from commercial chickens in South Africa, the promovenda generated complete genome sequence data for 178 mycoplasma isolates, identifying six species in the national flock, including Mycoplasma gallisepticum and M. synoviae, notifiable avian disease agents. Antimicrobial resistance profiles of 70 axenic isolates were correlated to genetic resistance markers, resulting in the identification of novel point mutations in M. gallinaceum and M. gallinarum associated with antimicrobial resistance. The promovenda furthermore assembled, annotated and published the first complete genome sequence of M. pullorum. Finally, 26 novel gene targets were identified in a whole genome comparison of 68 axenic mycoplasma isolates that will aid in the development of improved diagnostic assays and future vaccines. The sequencing database of 79 axenic isolates generated in this can be utilised for numerous future mycoplasma related gene studies.

Supervisor : Prof LCBGD Neves
Co-supervisor : Prof MC Oosthuizen
External co-supervisor : Dr V Delespaux (Vrije University Brussel, Belgium)
Internal examiner : Dr JJO Koekemoer
External examiner : Dr DW Berthier (CIRAD, France)
External examiner : Dr P Holzmuller (CIRAD, France)

Supervisor : Prof C Abolnik
External examiner : Prof MM Elgazzar (Iowa State University, USA)
External examiner : Prof M Gyuranecz (Institute of Veterinary Medical Research, CAR, HAS, Hungary)
External examiner : Prof MS Marenda (University of Melbourne, Australia)
In her thesis, Dynamics of highly pathogenic avian influenza outbreaks: incursion and emergence, the pro-
movenda demonstrated evolutionary relationships between Nigerian H5N1 viruses and Eurasian strains with evidence of co-circulating genotypes and emergence of reassortant strains. She also explored molecular changes involved in the emergence of highly pathogenic viruses from ostrich-origin low pathogenic H5/H7 progenitors in vivo. Deep sequencing data indicated progression towards high pathogenicity by the demonstration of increased basic amino acids at the connecting peptides of the hemagglutinin cleavage sites, and mutations in other gene segments. This work proves that should the low pathogenic viruses spill over from ostriches to chickens, the viruses can mutate into the highly pathogenic forms with a potentially devastating impact on poultry production as well as public health issues. The promovenda furthermore developed a standard operating procedure for the propagation of avian influenza virus in ostrich eggs.

Supervisor : Prof C Abolnik
Internal examiner : Prof FO Fasina
External examiner : Prof N Lewis (Royal Veterinary College, UK)
External examiner : Dr BZ Lönndt (Institute HVIVO, London, UK)

In this thesis, Development and implementation of the Rhinoceros DNA Index System (RhODIS®) for the forensic analysis and biological management of African rhinoceros, the development of a technique to individually identify rhinoceros horn through DNA profiling was described. The research utilised the DNA profile and biological data in a database, which includes representative samples of African white and black rhinoceros to support DNA profile matches in rhinoceros forensic cases. The research also applied these data to assist the biological management of extant rhinoceros populations. This study presents the data collected, technical and analytical methods used and shows the overall utility of the RhODIS® system as an important tool in protecting and sustaining rhinoceros populations. The results of this study received international recognition and established a precedent in the use of DNA forensics in wildlife crime cases.

Supervisor : Prof PN Thompson
External co-supervisor : Prof S O’Brien (St Petersburg State University, Russia)
Internal examiner : Prof E van Marie-Köster
External examiner : Prof BS Weir (University of Washington, USA)
External examiner : Dr OA Ryder (Institute for Conservation Research - San Diego Zoo, USA)
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