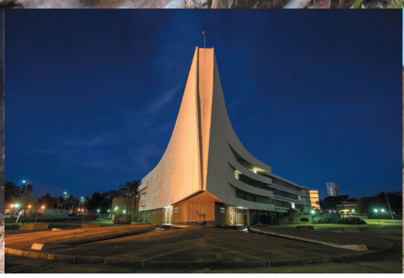
University of Pretoria

Animal & Zoonotic Diseases Institutional Research Theme

A talented mix of trans-disciplinary expertise tackling human and animal health issues – both nationally and globally.

2013



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The Institutional Research Theme Animal and Zoonotic Diseases (AZD-IRT) at the University of Pretoria draws upon a rich mix of trans-disciplinary research areas aimed at the management of a large variety of animal and zoonotic diseases of both national and global importance.

By incorporating some of the research strength and infrastructure of the Faculties of Veterinary Science, Health Sciences and Natural and Agricultural Sciences in a focused research theme, the University of Pretoria has positioned itself to play a leading role in the control of animal and zoonotic diseases. It will also enable the IRT to make a major contribution to the recently established Tshwane Animal Health Biocluster aimed at generating an enabling environment for the development of commercially viable tools and technologies for the control of some of South Africa's most important animal diseases. Several IRT associated projects are currently supported by the Tshwane Animal Health Biocluster.

Contents

"One Health" and the animal disease research chain

With its focus on zoonoses the IRT complements the University of Pretoria's "One Health". Umbrella. The "One Health" concept is based on the overlap between veterinary, human and ecological health sciences and its strength lies in the collaborative effort of multiple disciplines – working locally, nationally, and globally – to attain optimal health for people, animals and our environment.

This collaborative approach strengthens our research capability to address the complete animal *disease research chain.* This chain links three major research focus areas:

Disease Surveillance: Collection, analysis and interpretation of large volumes of disease-associated data that can be used in disease control and prevention.

Diagnostics: Identifying new and existing pathogens and disease patterns.

Disease Control: Preventative strategies, such as vaccines and therapeutic strategies.

By focusing on these research priorities the AZD-IRT aims to be recognised as having made an important contribution to the benefit of human and animal health and animal production in South Africa, Africa and the rest of the world.

Animal diseases

Animal health has undergone an almost unprecedented increase in importance in the last century and is today inextricably linked to our local and global economy as well as societal issues such as public health, food and food security. The lack of control of animal diseases such as avian influenza, foot-and-mouth disease, bluetongue and bovine tuberculosis as well as ticks and tick-borne diseases such as heartwater and anaplasmosis will a hugely negative economic and social impact.

Zoonotic diseases

Many diseases are not restricted to a single animal species. We are witnessing the increased emergence of dangerous animal and trans-boundary diseases, specifically zoonotic diseases. Zoonoses are caused by microbes (largely bacteria, viruses or protozoa) that are transmitted from vertebrate animals to humans, regularly resulting in dangerous outbreaks of disease.

What are Institutional Research Themes?

The University of Pretoria is consolidating its traditional research strengths by supporting the development of multidisciplinary Institutional Research Themes. IRTs are chiefly directed towards fostering a more collaborative approach internally, as well amongst other academic institutions, private enterprises and governmental agencies. By embracing a knowledge-sharing philosophy, IRTs are ideally placed to promote research on the world's most pressing social, economic and environmental problems.

On average, the emergence of new diseases occurs every eight months, of which more than 70 per cent originate as animal diseases.

Animal hosts often act as reservoirs for pathogens, or transmit the disease to other species. Familiar examples of zoonoses include anthrax, rabies and yellow fever. Over 60 per cent of all known pathogens affecting humans are zoonotic. On average, the emergence of new diseases occurs every eight months, of which more than 70 per cent originate as animal diseases.

Capacity development

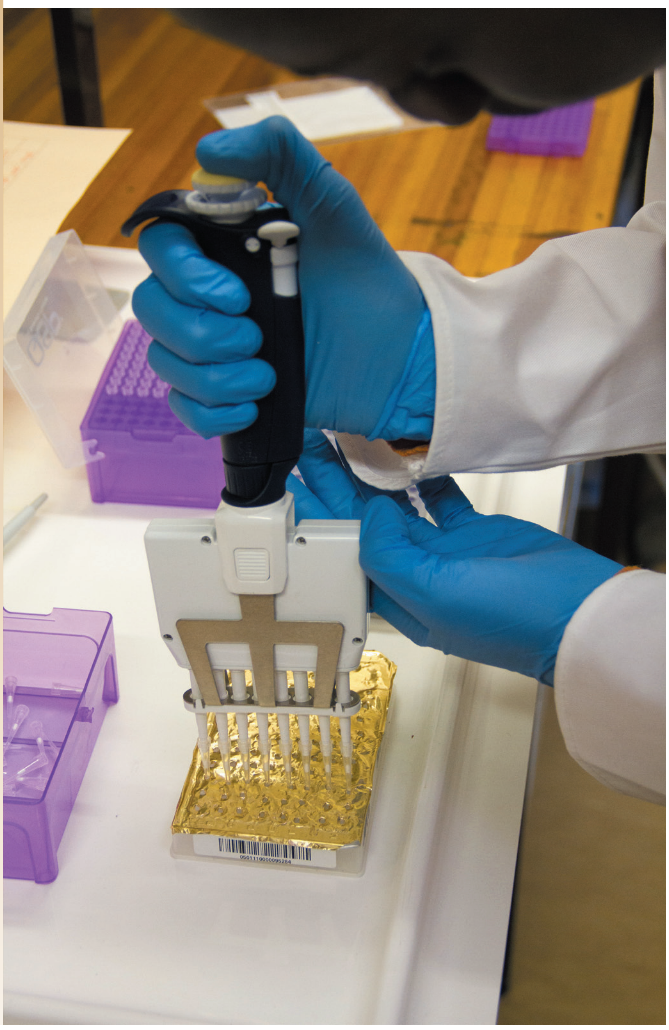
Apart from its focus on animal and zoonotic diseases the IRT is seen as an important contributor towards the University of Pretoria's role in building research capacity in this field.

There is therefore a large focus on supporting start-up projects that play a role in postgraduate MSc and PhD student training. The IRT also aims to use its funding to leverage external funding that will significantly expand our ability to expand our role in capacity development and research.

Research Focus Areas

Surveillance and Control of Vector-Borne Zoonotic Viruses

The surveillance and diagnostics of emerging zoonotic pathogens is an essential component of the control of zoonotic diseases. In the Zoonoses Research Unit in the Faculty of Health Sciences the principal investigators Prof Marietjie Venter, Prof Robert Swanepoel and Prof Leo Braack are focused on a variety of questions associated with disease surveillance and control of a wide range of zoonotic pathogens such as West Nile Virus, Rift Valley fever virus and Crimean-Congo haemorrhagic fever virus as well as viruses such as avian influenza that are transmitted directly from animals to humans.



Surveillance of pathogens associated with neurological disease

A primary research area concerns surveillance of unexplained fevers, neurological, and haemorrhagic syndromes and respiratory illness in humans, farm and wild animals – and tracking down causative agents. The research provided the first evidence of West Nile virus (WNV) as a cause of neurological disease in humans and animals in South Africa and revealed that veterinarians and other individuals with frequent exposure to livestock are at increased risk of infection. This focus has been expanded by an internationally collaborative project to evaluate different lineages of West Nile Virus vaccine candidates.

New tool to identify zoonotic pathogens

An important contribution to disease surveillance and pathogen discovery has been made by the development of a range of specialized molecular and serological diagnostic tools, the development of one of these is currently supported by the Tshwane Animal Health Biocluster. Innovative diagnostic tests have been established, including a test to identify zoonotic causes of meningitis in South Africa as well as tests that support the detection of zoonotic viruses associated with fatal infections in livestock and wildlife.

In 2011 a state-of-the-art BSL-3 laboratory was acquired to facilitate the study of zoonotic viruses (see page x). The ZRU maintains strong links with the National Institute of Communicable Diseases (NICD) in Johannesburg and several overseas collaborators, including the Global Disease Detection Programme of the Centers for Disease Control and Prevention in Atlanta.

Facilities

Biosafety Level 3 (BSL-3) laboratory

The containerized BSL-3 laboratory, erected on Prinshof Campus.

Zoonoses often involve dangerous diseases such as SARS, avian influenza, Marburg or Ebola fevers. Their diagnosis and careful study are critical to disease control and depend to a large degree on molecular techniques to detect viral genetic material in a variety of samples. Effective disease control is therefore dependent on our ability to grow pathogens under safe conditions, which are necessary to facilitate pathogenicity and vector transmission studies.

These requirements motivated the University of Pretoria to acquire (with the support of the Ministry for Higher Education) a Biosafety Level 3 (BSL-3) laboratory that can be used for work on infectious agents.

The BSL-3 laboratory plays a critical role in several AZD-IRT projects and programmes across three different faculties. The laboratory enables us to grow, characterize and utilize a live virus, for example, in preparing immunological reagents for use in diagnosis at different stages of the progression of a disease. The laboratory has been operational since 2011, and is staffed by members of the Zoonoses Research Unit.

BSL-3 laboratory features

The laboratory possesses a sophisticated ventilation system. Air pressure in the various rooms is maintained in a negative cascade; when doors are opened, air leaks towards the room where the most dangerous procedures are performed. Both inlet and exhaust air is passed through highly efficient filters to prevent the accidental release of dangerous infectious agents into the environment. Moreover, work with the agents is performed inside safety cabinets which themselves have a filtered air supply to prevent escape of infectious agents into the atmosphere.

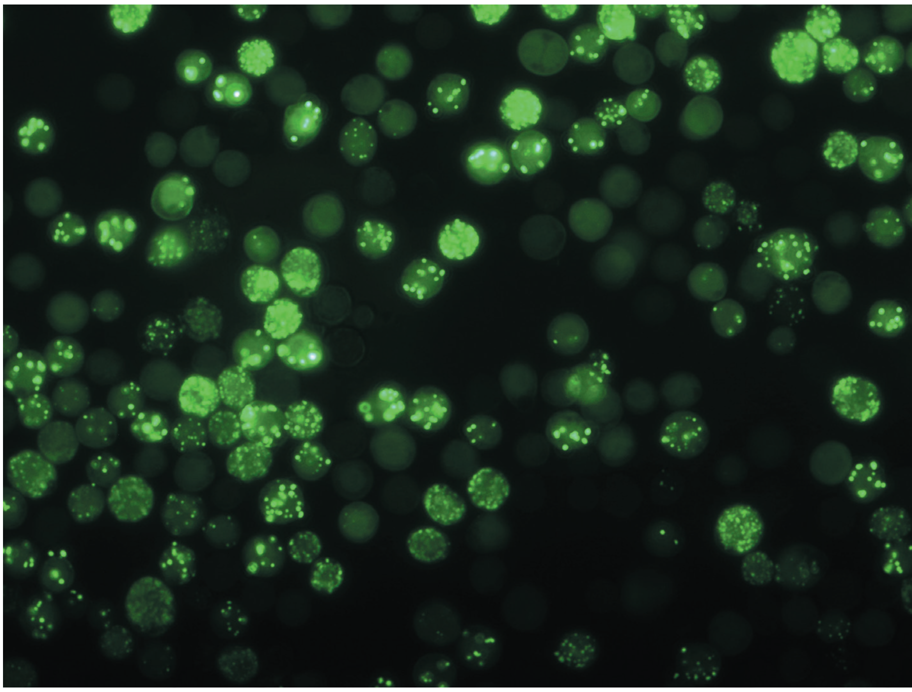
The completed laboratory adjacent to the Pathology Building. Air conditioning condensers and the effluent decontamination system are housed under a separate roof.

The double-door autoclave, pass-box and dunk-tank in the anteroom of the BSL-3 laboratory permit safe passage of materials in and out of the laboratory.

BSL-3 workers wear protective clothing, including gloves, gowns and battery driven positive pressure respirators which pump filtered air into a hood worn over the head to prevent any possible accidental inhalation of infectious organisms. Effluent from the drains is treated with heat and/or disinfectant to render it safe, while all solid waste is autoclaved before removal.

Clad in protective clothing, Professor Marietjie Venter (Zoonoses Research Unit, Department of Medical Virology) examines a sample in the Class 3 biosafety cabinet inside the laboratory.

The control of bluetongue and African horse sickness



Both African horse sickness and bluetongue are diseases of global importance. The spread of bluetongue to the Northern parts of Europe has raised international alarm bells and increased a wide-ranging research focus on aspects that will enable us to control these diseases. In South Africa African horse sickness has a major impact on the horse industry and especially so because it affects the international trade and transport of horses. The research of the principal investigators in the field of African horse sickness and bluetongue is focused on the broad questions related to viral assembly, viral pathogenesis and the development of new vaccines.

A reverse genetics approach to study and control disease

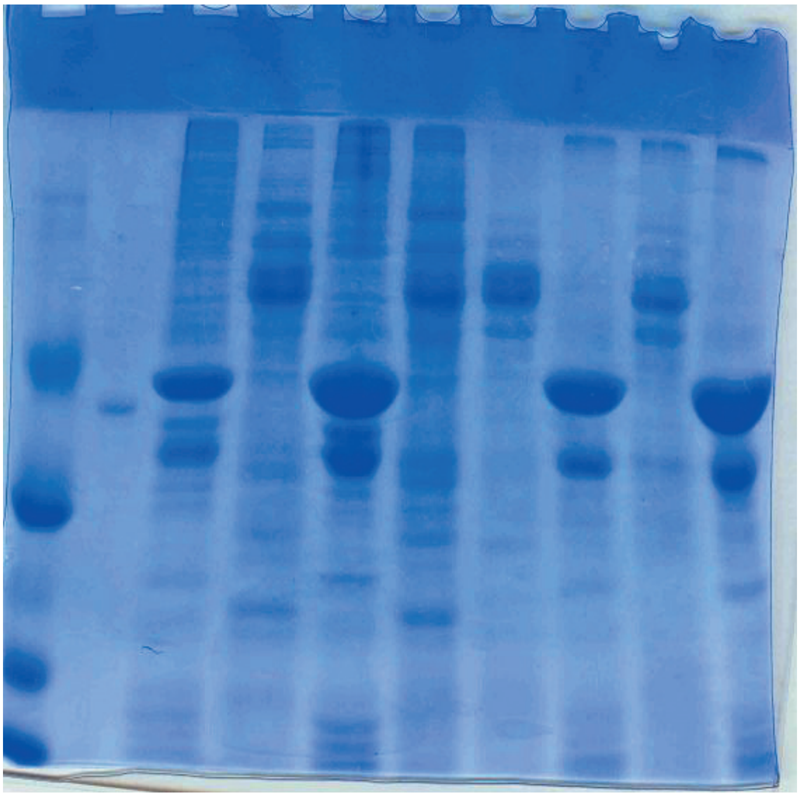
The research of Prof Jacques Theron is largely focused on the development of a reverse genetics platform for African horse sickness virus. The project is supported by Tshwane Animal Health Biocluster and is aimed at enabling the selective genetic modification of the viral genes followed by the reconstitution of infectious recombinant virus. This ability allows researchers to study the role of the different virus proteins in virus replication, virulence and immune protection. The strategy is fundamental to the development of a new generation of designer vaccines by generating viruses

with modified pathogenic properties. The research is further complemented by RNA interference technologies to study the function of viral proteins.

This research complements the research of Dr Vida van Staden, focused on the role of AHSV non-structural proteins in the virus replication and virus-cell interaction. A variety of technologies, including electron microscopy and confocal microscopic studies and the use of fluorescently tagged proteins are used to address how these proteins are transported and interact in virus-infected cells.

Bluetongue virus transmission through the placenta

The research of Prof Estelle Venter has a large focus on BTV. It has been established that the wild-type virus can infect goats via the transplacental route – which is one possible method by which the virus overwinters. The group is also participating in an internationally collaborative project to study cell-virus interaction, using BTV reassortants produced via exchange in cell cultures or reverse genetics. This project also forms part of the Tshwane Animal Health Biocluster of funded projects.



The control of ticks and vector-borne protozoal and rickettsial diseases

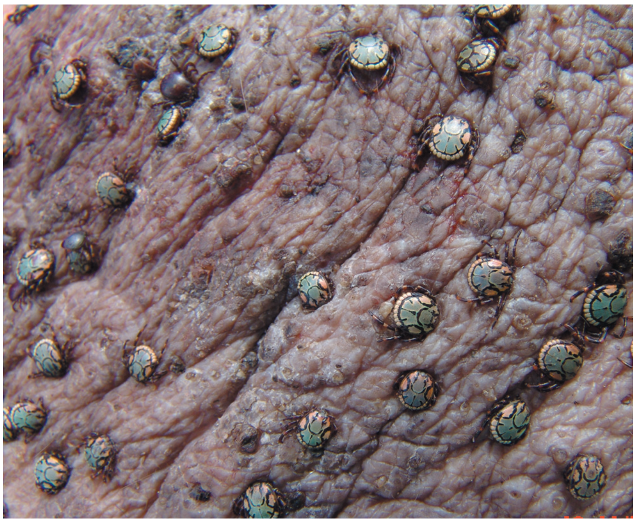
Ticks and tick borne diseases place a major constraint on livestock production in South Africa. Currently, a provisional estimation of revenue lost due to cattle losses range between R1.3 billion and R3.7 billion per year. The principal researchers in this field of study, Dr Christine Maritz-Olivier and Prof Marinda Oosthuizen, are focused a range of initiatives that include an anti-tick vaccine programme, a vector control programme, as well as evaluating various components that play a role in disease control. Vector-borne diseases which affect domestic animals and wildlife in South Africa such as Babesiosis, Theilerosis and Anaplasmosis are studied.

A novel approach to tick control

The tick research programme of Dr Maritz-Olivier investigates the genetic diversity and current acaricide resistance status of *Rhipicephalus* ticks in the South African region. Ongoing analyses of five acaricide resistance genes and eight microsatellites form part of a large-scale screening of *Rhipicephalus* ticks. These studies have provided researchers with an understanding of the parasite-host-pathogen interactions and supported the development of economically viable tick control measures. The vector control programme has been expanded. New research initiatives include studies to evaluate the mode of action of different *Babesia* species using functional and comparative genomics strategies. This research will prove critical for future drug design and animal health.

Anti-tick vaccine programme

The research group of Dr Maritz-Olivier is currently identifying novel anti-tick vaccine candidates via a range of different strategies that include transcriptome analyses, reverse vaccinology and RNA interference. The programme is also supported by the Tshwane Animal Health Biocluster and has already yielded two promising vaccine candidates which are in various stages of patenting and animal testing trials. Additional candidates have been identified by a new reverse vaccinology approach, expressed by a recombinant DNA strategy in quantities suitable for cattle vaccine trials.




Vector-borne disease programme

The research of Prof Oosthuizen is focused on a vector-borne diseases programme that involves the molecular characterisation of parasites, mostly through the 18S ribosomal RNA gene. Significant improvements in molecular diagnostic assays promise to add an important contribution to the detection of *Theileria parva* in buffalo and cattle in South Africa. Buffalo are considered to be a natural reservoir of *Anaplasma* infections and may play an important role in the spread of the disease.

The programme has already yielded two promising vaccine candidates which are in various stages of patenting.

Rickettsia research

Heartwater is a serious disease found in domestic and some wild ruminants throughout sub-Saharan Africa, and in some parts of the Caribbean. The disease is caused when the rickettsia *Ehrlichia ruminantium* is injected by the bite of an infected tick. Outbreaks often result in serious losses to farmers, who can lose up to 80-95 per cent of their animals. The search for a vaccine stretches over many decades, thus far with limited success – although the whole rickettsia genome was recently sequenced and annotated.

Epidemiology and control of Rabies and other Viral Zoonoses – particularly those associated with bat species.

This program incorporates research on rabies and rabies-related viruses and an array of viruses associated with bats. The research programme, directed by Prof Louis Nel, Prof Wanda Markotter and Prof. Darryn Knobel is aimed at the continual improvement of the capability to detect, control and treat these viruses and the corresponding diseases. It involves viral epidemiology, viral evolution, pathogenicity and mechanisms of infection, tropism and host specificity. There is a large focus on the genetic diversity and pathogenicity of the disease-causing organisms and their main vectors. The strength of this group is characterized by substantial local and international collaborations.

Rabies control and surveillance

Rabies remains an important disease in South Africa with a particular high impact on rural communities. It is a high priority to get a complete picture of the epidemiology of this disease in order to control the spread of disease. The data acquired is used in disease modelling and a range of other control strategies. There are equally important question to be addressed with respect the source of rabies and rabies-related viruses. Rabies in domestic dogs has been eliminated in most of the developed world, thanks to extensive vaccination and dog movement restrictions. However, rabies is a neglected disease in less developed areas where domestic dogs remain the principle vectors, contributing to more than 95 per cent of human rabies cases.

Prof Louis Nel directs a dog rabies elimination programme, launched by the global Alliance for Rabies Control who secured a five-year sponsorship from the Bill and Melinda Gates Foundation. KwaZulu Natal has been selected as the site for this programme which is based on a strong partnership with the provincial government. It includes vaccine development, dog ecology studies, epidemiology, phylogeographical modeling and new platforms for diagnosis. We believe that rabies control not only involves vaccination but also depends on improving the health of animals in these communities using a combination of vaccination campaigns, sterilisation and general animal health activities.

Research on zoonotic and other animal pathogens in bats has identified several novel viral and other pathogens in a variety of bat species from southern Africa.

Further vaccine initiatives include a collaborative project with Thomas Jefferson University involving a reverse genetic system in a study of lyssavirus pathogenesis, as well as the development of a recombinant rabies virus vaccine.

Dog population demographics and rabies control

Dog population demographics (birth and death rates) have a major influence on the long-term effectiveness of rabies vaccination efforts, because coverage levels decline as vaccinated dogs die and, more importantly, as new dogs are born. To develop more effective rabies control strategies, Prof. Darryn Knobel and his research team are monitoring birth and death rates in a population of 1,000 dogs in a rabies-affected community. This study site, with its well-characterised population of dogs and engaged owners, is used as a platform for an IRT-supported study to test the effectiveness of an injectable contraceptive vaccine in reducing the birth rates of dogs.

Studying zoonotic viruses in bats

Bats play host to several serious diseases, including rabies and related viruses. Under the leadership of Prof. Markotter several novel viral and other pathogens in a variety of bat species have been identified by using a multidisciplinary approach in the study of the emergence of disease amongst populations of cave roosting bats in South Africa. The study of these viruses requires high containment facilities, such as the Biosafety Level 4 facility at the National Institute of Communicable Diseases in South Africa, the only one of its kind in the region and this programme is in collaboration with this Institute. Current and future research aims to expand surveillance and to diagnose and detect novel pathogens, determine their pathogenic effect in the host species and also to study host ecology factors that may influence disease emergence. An IRT-supported programme has also been launched to generate cell lines specific for bat species. Such cell lines will facilitate improved isolation and propagation of these viruses and as such support virus characterization and studies of viral pathogenicity.

The Control of Bacterial and Nematode Zoonotic Diseases

This programme involves a number of principal investigators with a strong focus on a variety of bacterial zoonotic diseases such as bovine tuberculosis and anthrax that impact on human, wildlife and livestock. There is also a focus on nematode associated diseases and pathogens. The overall emphasis is on innovative new strategies to improve the capability to develop, detect and control these diseases.

A trail of molecular fingerprints

A molecular fingerprinting strategy is used by Dr Henriette van Heerden to determine the prevalence and distribution of bovine tuberculosis, brucellosis and anthrax. An important aim is to trace the origin and routes of transmission of a specific outbreak strain within and between wildlife, livestock and humans. A range of different diagnostic tests, including those for bovine tuberculosis and brucellosis were optimised and validated and are used in disease surveillance studies in Hluhluwe-Umfolozi National Park.

In an AZD-IRT-supported research project of Dr Marthie Ehlers a molecular strategy is used to address the prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* genotypes in the pig population and their human contacts in the Gauteng area. This bacteria is typically considered a hospital-related problem, but has recently emerged as an important community associated pathogen.

In another IRT funded project Dr Pamela de Waal is making a study of the genetic variation of the nematode parasite *Spirocerca lupi* which affects dogs. A host of genetic markers for quantification of genetic diversity and specific detection in the host organism have been developed.

Developing vaccine and other control strategies

Another part of Dr van Heerden's programme is focused on exploring different anthrax vaccine strategies in humans and cattle. This study, supported by Tshwane Animal Health Biocluster, includes studies on immunogenicity and protectivity of a live spore anthrax vaccine in comparison to recombinant peptide and DNA vaccines in goats. Similarly, the role and impact of non-tuberculous mycobacteria on the immune responsiveness of cattle and buffalo to BCG vaccination are under investigation.

Apart from researching vaccines as a control strategy, Dr Lyndy McGaw is involved in studying anti-mycobacterial compounds in plant extracts in an AZD-IRT funded project, conducted in collaboration with the Department of Plant Sciences. Such compounds may reveal different mechanisms of action, or could complement current pharmaceuticals.

Controlling Pathogens at the Wildlife and Livestock Interpahse

The control and study of pathogens that operate at the wildlife:livestock interphase is of large importance in South Africa with its focus on game and livestock farming. In the Molecular Epidemiology & Zoology group in the Faculty of Natural and Agricultural Sciences Prof Mandy Bastos makes use of molecular and modelling techniques to determine the prevalence, course and diversity of the pathogens that put animals at risk.

Targeting diseases with PCR-based diagnostic methods

In order to identify and characterize the pathogens that play a role in these zoonoses the research group has development of a variety of PCR-based diagnostic methods that enable the rapid detection and identification of the disease agents and their hosts. This data is applied in epidemiological studies, to refine diagnostics and to guide the process of determining appropriate vaccine strains and disease control policies.

This strategy has been applied in the recent characterisation of a herpesvirus associated with clinical malignant catarrhal fever. Results revealed the presence of two viral lineages in South Africa. This is important in vaccine strain selection and the strategy to control this disease in South Africa.

Another focus area is on zoonoses amongst murid rodents. The accurate identification of host species is particularly important because of the large number of cryptic murid rodent species that occur in South Africa.

MRSA is typically considered a hospital-related problem, but has recently emerged as an important community associated pathogen.

The control of Rift Valley Fever

Rift Valley Fever remains an important zoonosis in South Africa and the rest of Africa, A research group headed by Prof Koos Coetzer in the Faculty of Veterinary Sciences in the Department of Diseases of Veterinary and Tropical Diseases is conducting a comprehensive research programme with national and international partners on the epidemiology, diagnosis and prevention of Rift Valley fever in domestic and wild ruminants in southern and eastern Africa.

The control of Poultry Diseases in South Africa

Poultry diseases are a continuous and dynamic threat to poultry production in South Africa, be it through introduction of new strains or diseases from abroad or natural mutation of enzootic populations. Several of these pathogens have zoonotic potential. Periodic outbreaks of the OIE-notifiable Newcastle disease and highly pathogenic avian influenza cause high mortalities whereas others pathogens such as infectious bronchitis virus and infectious bursal disease virus cause milder yet economically significant production losses. The problems associated with these diseases are addressed by Prof Celia Abolnik, recently appointed Chair in Poultry Health and Production in August 2012.

Industry focused applied research

The research into poultry diseases is mostly applied, and driven by the needs of the industry. The current research focus includes developing diagnostic tests in line with international advances in technology, conducting surveillance, characterising pathogens at a genetic level and testing vaccines.

Basic research

However, basic science into poultry pathogens is equally important and contributes to global knowledge. The research used to come to a better understanding of the pathogens involved includes cutting-edge technologies. Ultra-deep sequencing is used to investigate how these pathogens cause disease, mutate, evade host responses and interact with their host.

Three different projects of Prof Abolnik are supported by the Tshwane Animal Health Biocluster .

Vaccines Research Summary

- **West Nile Virus:** A vaccine trial in mice demonstrated cross-protection of a West Nile Virus lineage 1 commercial vaccine for use against lineage 2 in horses. Collaborative projects are addressing local development of subunit vaccines for WNV.

- **African Horse Sickness Virus**: Current research is focused on the development of a reverse genetics system for AHSV, where viral proteins can be modified and mutated by design. This enables our researchers to address questions with respect to viral protein function.

- **Anti-tick Vaccine Programme**: Novel anti-tick vaccine candidates are being identified via a range of different strategies that include transcriptome analyses, reverse vaccinology and RNA interference. The programme has already yielded two promising vaccine candidates which are in various stages of patenting.

- **Rabies Control and Surveillance:** Vaccine initiatives include a collaborative project with Thomas Jefferson University, involving a reverse genetic system in a study of lyssavirus pathogenesis, as well as the development of a recombinant rabies virus vaccine.

- **Bacterial and Nematode Zoonotic Diseases:** Different vaccine strategies in humans and cattle are being explored, including studies on immunogenicity and protectivity of a live spore anthrax vaccine in comparison to recombinant peptide and DNA vaccines in goats.

More information:

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