Southern African Society for Veterinary Epidemiology & Preventive Medicine

PROCEEDINGS

FOR THE

RuVASA & SASVEPM Congress

Birchwood Hotel
Boksburg
Gauteng
18-20 June 2018

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SASVEPM Congress

The Southern African Society for Veterinary Epidemiology and Preventive Medicine (SASVEPM) strives to enhance veterinary epidemiology and preventive medicine in Southern Africa, adapt the epidemiological discipline to address regional diseases and challenges and build or strengthen the network and capacity of Southern African veterinary epidemiologists.

In line with these goals, we are proud to be hosting the 2018 annual SASVEPM congress with the Ruminant Veterinary Association of South Africa (RUVASA). This is one step further toward strengthening the role and relevance of epidemiology in the Southern African region through the integration of existing networks established for animal disease prevention, detection and control.

This year's three day congress presents exciting new epidemiological, diagnostic, and experimental work from Southern Africa. We hope to offer RUVASA members an insight to investigations in species other than ruminants, and expose SASVEPM members to the context and progress of Ruminant health issues.

SASVEPM has also endeavored to keep abreast with critical health events affecting South Africa, such as the impact of Listeriosis on Public Health and the outbreak of emergent Highly Pathogenic Avian Influenza H5N8 in the Southern Africa Region. The morning of Day 1 will address HPAI and Day 2 of the congress will be focused on the outbreak of Listeria monocytogenes ST 6.

The 2017/8 EXCO would like to thank all presenters and organizers of this year's congress for their hard work, commitment and contribution to the ongoing success of Epidemiology and Ruminant Medicine in the Southern African region.

DAY ONE- 18 JUNE 2018			
Session	***Time***	***Title***	***Presenter***
	07:00- 08:00	***Registration***	
Opening	08:00- 08:15	Opening address	SASVEPM President
	08:15- 08:45	Keynote 1: Veterinary field Epidemiology in South Africa- From Arnold Theiler to the internet of things	McCrindle CME
HPAI	08:45- 09:15	Keynote 2: A single vaccination with recombinant H5 antigen produced in insect cells induced 100% protection against an HPAI H5N8 isolate from South Africa	Mundt E
	09:15- 09:40	Close to Real-Time conversion of basic data into valuable information using open-source technologies: The South African HPAI outbreak as an example	Grewar JD
	09:40- 10:05	Spatial Epidemiology of Highly Pathogenic Avian Influenza H5N8 outbreaks during 2017 Mpumalanga, South Africa	Dongo JC
	10:05- 10:35	**Tea/Coffee**	
Equines	10:35- 11:00	The 2015/2016 trade network of live equids destined for South Africa	Grewar JD
	11:00- 11:25	A freedom of disease survey: African Horse Sickness virus in South Africa's surveillance zone in 2017	Grewar JD
	11:25- 11:50	Middelburg and Sindbis Alphaviruses as a cause of febrile and neurologic disease in horses and humans in South Africa	Fourie I
	11:50- 12:15	Taylorella spp. in South Africa: The Saga continues	May CE
	12:15-12:40	Failure to detect equid alphaherpesvirus 1 DNA in thoroughbred placentae and healthy new-born foals	Brown LJ
	12:40- 13:05	Socioeconomic impacts of working horses in urban and peri-urban areas of the Cape Flats, Cape Town, South Africa	Grewar JD
	13:05- 14:00	**Lunch**	
Data analysis methods	14:00- 14:25	Analysing Production and financial data from farmers to identify sustainable poultry enterprises in a resource constrained rural poultry Sub-sector of Eastern Zambia	Mubamba C
	14:25- 14:50	Application of Spatial Scan statistics and Spatial Empirical Bayesian smoothing in investigation of Spatial patterns of Diseases.	Qekwana DN
	14:50- 15:15	Using prevalence data to estimate seroconversion and sero-reversion rates of Rift Valley Fever virus in an endemic area	Thompson PN
Bovines	15:15- 15:40	Isolation of Brucella Melitensis Biotypes 2 and 3 from slaughter cattle in South Africa	Kolo FB
	15:40- 16:20	**Tea/Coffee**	
	16:20- 16:35	Veterinary Strategy Update	Modisane BM
	16:35- 16:50	NAHF Address	Vervoot P
	16:50- 17:10	SAVC address	Marwick C

DAY TWO- 19 JUNE 2018 (Listeriosis)			
Session	***Time***	***Title***	***Presenter***
Session 1	08:00- 08:30	Link between *Listeria monocytogenes* and processed meats	Elna Buys
	08:30- 09:00	The Listeriosis outbreak in South Africa: Useful microbiological testing for *Listeria monocytogenes*	Lucia Anelich
	09:00- 09:30	Consumer rights and Civil Class Action cases involving Listeria, and the role this could possibly play in the South African response to *Listeria monocytogenes* detected in processed food	Januz Luderek
	09:30- 09:40	Questions	
	09:40- 10:10	**Tea/Coffee**	
Session 2	10:10- 10:35	The listeria hysteria	Christo Labuschagne
	10:35- 11:00	Listeria in the 21st century- Behaviour in food processing and food distribution in the "global village"	Georgina Pondayi
	11:00- 11:25	The role of education, prevention and end point testing in detecting and controlling *Listeria monocytogenes*	Elna Buys
	11:25- 11:50	Persistence of different *Listeria monocytogenes* strains from processing plants	Thulani Sibanda
	11:50- 12:15	Establishing microbiological criteria for meat products in South Africa (DAFF)	Kudakwashe Magwedere
	12:15- 12:45	Panel discussion: What now for industry? Roles and responsibilities of stakeholders	
	12:45- 13:45	**Lunch**	
Session 3	13:45- 14:05	The role of Environmental Health Practitioners in the South African *Listeria monocytogenes* outbreak detection and response	Rina Nel
	14:05- 14:25	The role of National Institute for Communicable Diseases in the South African *Listeria monocytogenes* outbreak detection and response	Kerrigan McCarthy
	14:25- 14:45	Gauteng provincial health response to *Listeria monocytogenes* outbreak in South Africa	Mary Madaure
	14:45- 15:05	Risk-based approaches to managing food safety in South Africa: a scientific review with reference to *Listeria monocytogenes* outbreak in South Africa	Charles Katsande
	15:05- 15:25	Integrating provincial food safety functions under the One-Health umbrella	Yemi Akerele/ Shepherd Kamudyariwa
	15:25- 15:45	Panel discussion: Role of epidemiology in the early detection and rapid response to foodborne outbreaks of public health importance	
	15:45- 16:15	**Tea/Coffee**	
	16:15- 17:15	SASVEPM AGM	
	19:00	***Gala Dinner***	

DAY THREE- 20 JUNE 2018			
Session	***Time***	***Title***	***Presenter***
Ruminants	08:00- 08:25	Cattle production management practices predisposing animals to the incidences of reproductive conditions is small scale farming	Molefe K
	08:25- 08:50	Prevalence of Campylobacteriosis and Trichomoniasis in communal cattle of Umgungundlovu, Kwazulu-Natal	Badenhorst S
	08:50- 09:15	Risk Factors for Bovine Tuberculosis in cattle and communal farmers living at the wildlife-Livestock-Human interface in Kwa-Zulu Natal, South Africa	Sichewo PR
	09:15- 09:40	Endemic circulation of Rift Valley fever virus in far Northern Kwazulu-Natal	Van den Bergh C
	09:40- 10:05	Temporal patterns of Anthrax outbreaks and cases among livestock in Lesotho over a period of Eleven years (2005-2016)	Lepheana RJ
	10:05- 10:30	Development of improved molecular assays for epidemiology characterisation of Shuni Virus	Motlou TP
	10:30- 11:00	**Tea/Coffee**	
Canine/ Rabies	11:00- 11:25	Multinomial logistic regression in Infection: Application for Diagnosis of canine clinical cases of multi species staphylococcus infection	Qekwana DN
	11:25- 11:50	Validation of an Indirect Immunoperoxidase test rabies virus in domestic and wildlife species in South Africa	Janse van Rensburg DD
	11:50- 12:15	A bioeconomic model for the optimization of local and region canine rabies control	Kotze J
Wildlife	12:15- 12:40	Pathology Survey of diseases of African Buffalo in South Africa	Mitchell E
	12:40- 13:05	How sure are you of that result?	Cloete AS
	13:05- 13:20	***Closure of congress***	
	13:20- 14:20	**Lunch**	

Session Chairs		
Day One- 18 June 2018	Session 1- HPAI	Dr Didi Janse van Rensburg
	Session 2- Equines	Dr Nelson Matekwe
	Session 3- Data Analysis	Dr Wonderful Shumba
Day Two- 19 June 2018	Session 1	Dr Krpasha Govindasamy
	Session 2	Dr Leana Janse van Rensburg
	Session 3	Dr Charles Katsande
Day Three- 20 June 2018	Session 1- Ruminants	Dr Grietjiede Klerk
	Session 2- Wildlife	Dr Vashnee Govender

DAY 01

DAY 02

DAY 03

Veterinary Field Epidemiology in South Africa – from Arnold Theiler to the Internet of Things Veterinary

McCrindle, C.M.E. [1*]

Veterinary Epidemiology is the study of the causes of diseases in animal populations. During the 19th Century epidemics of animal diseases crippled the economy and caused immense suffering in both animal and human populations in South Africa. Some of the diseases, like African horse sickness, blue tongue, rabies, anthrax, black quarter, redwater, East Coast fever and nagana had spilled over from indigenous animals; while others, like strangles, glanders, brucellosis, bovine tuberculosis, lungsickness (contagious bovine pleuropneumonia) and rinderpest were imported. Finding the causes of these epidemics, mainly through field epidemiology, facilitated their eventual decline. David Bruce, who was in the Royal Army Medical Corps stationed in Pietermaritzberg, identified *Trypanosoma brucei* as the cause of Nagana, after discovering the cause of undulating fever (Brucellosis) in Malta. Robert Koch, whose field epidemiology had enabled him to culture Anthrax and propound his postulates, visited Pretoria in 1896, but failed to stop the Rinderpest outbreak. Louis Pasteur came from France to discuss the idea that filterable agents smaller that bacteria were responsible for rabies and possibly other diseases like horse sickness and blue tongue in South Africa. After dedicating a decade of his life to field epidemiology, Arnold Theiler developed a diagnostic and vaccine production centre and also initiated the first South African veterinary faculty in the first decade of the 20th Century. The University of South Africa subsequently introduced a curriculum for Animal Health Technicians in the late 20th Century. However, despite huge advances in technology; African horse sickness, blue tongue, rabies, tuberculosis, redwater and brucellosis remain endemic; and outbreaks of other animal diseases like avian influenza still impact on the economy and animal welfare, while zoonoses like listeriosis compromise human health as well as the economy. This paper discusses the possibility of including advances in traceability, large data-base management and the internet of things, to facilitate field surveillance and monitoring of animal diseases and zoonoses in the 21st Century.

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Gutsche Thelma 1979 There was a man – the life and times of Sir Arnold Theiler K.C.M.G. of Onderstepoort . Howard Timmins, 43 Shortmarket Street, Cape Town, South Africa.

Zhi Li, Guo Liu, Layne Liu, Xinjun Lai, Gangyan Xu 2017 IoT-based tracking and tracing platform for prepackaged food supply chain. Industrial Management & Data Systems, 117 (9): 1906-1916, https://doi.org/10.1108/IMDS-11-2016-0489

Close to Real-Time Conversion of Basic Data into Valuable Information using Open-Source Technologies: The South African HPAI Outbreak as an Example

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Open-source software is computer software that is has freely available source code which can be utilised to improve open collaboration. Within the scientific community at large statistical software such as R has become the core of data manipulation and visualisation, with any developer having the ability to make packages that can be utilised in the real time analysis of data. Open-source software is not limited to statistical analysis, and of relevance to this report, there is database (in this case PostgreSQL) and spatial GIS systems (PostGIS and qGIS/qGIS sever) that are also freely available to utilise. Cloud computing has provided a platform for the dissemination of information in resource constrained environments where server acquisition and maintenance functions are not sustainable and require significant upskilling of employees. While cloud computing is not free it does allow a subscription type service of server space in a very controlled and secure environment, with no penalty when said services are no longer required.

Outbreaks of disease, such as the highly pathogenic H5N8 outbreak that occurred in South Africa in 2017, result in significant stress to both the industry affected and the authorities tasked with controlling the disease. In such events it has been our experience that the availability of basic epidemiologic information in near real-time greatly assists stakeholders in reporting and making scientifically sound decisions. The term 'basic' epidemiologic information is also a misnomer here. Often in an outbreak the most valuable information is sourced from the most basic of outbreak investigation data – in this case simply when each infected property (IP) became infected (i.e. the temporal variable) and secondly where each IP is situated (i.e. the spatial variable). These data are obtained from publically available OIE reported information. Putting these two variables together one is able to assess the outbreak in near real-time in a spatial and temporal context which provides information on:

- Type of epidemic as it evolves
- Spread of epidemic and risk of new areas being infected
- Potential of determining spatial and temporal end-points of infection
- Determination of clustering which assists in disease control policy for infected areas

In this report we show how cloud computing, combined with R (and its associated packages) and making use of PostgreSQL and qGIS and their relevant server capabilities, can create an output that is close to real-time and converts basic space time data into valuable epidemiologic information to be used by both industry and control authorities.

HPAI One Health Awareness Days - Effective Training of Multidisciplinary Stakeholders

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In 2016, Gauteng Veterinary Services adopted the "One Health Initiative" into the Veterinary Strategy. The newly formed provincial One Health Management Team, comprising 20 individuals representing governmental and non-governmental disciplines of Veterinary Science, Public Health, Medicine, Social Science, Wildlife and Epidemiology, responded to the South African outbreak of HPAI (Highly Pathogenic Avian Influenza) H5N8, detected in June 2017. From June to November, Gauteng province experienced 13 outbreaks that included outbreaks in poultry facilities, wild water birds and outbreaks in zoo facilities. A need was identified by the One Health Management team to increase the capacity of stakeholders responsible for HPAI detection and control, to respond appropriately to HPAI outbreaks in poultry facilities and facilities housing protected, endangered or critically endangered avian spp. The team arranged two events to train multidisciplinary stakeholders to detect and respond to HPAI, using a One Health Approach. The first day, held on the 3rd November 2017, simulated an outbreak of an emergent zoonotic strain of HPAI H5N8 on an emergent poultry farm. The second event, held on the 14th November 2017, focussed on an outbreak in an open air enclosure in a zoo, housing exotic, endangered birds. Both days were evaluated using a pre and post knowledge, perceptions and attitudes questionnaire. Products that were created for the day, including training manuals, brochures, posters and mugs will be illustrated. Critical gaps in knowledge and perceptions of risk will be described as well as the baseline knowledge of the different disciplines in respect of HPAI detection and response.

Spatial Epidemiology of Highly Pathogenic Avian Influenza H5N8 Outbreaks During 2017 in Mpumalanga, South Africa

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June to August 2017 there was ten outbreak clusters of highly pathogenic avian influenza H5N8 (HPAI H5N8) affecting commercial and back yard poultry and wild birds on the highveld of Mpumalanga, South Africa. The spatial distribution was described and compared with the avian influenza risk map of G. S. Cumming and others, 2008:26. Proximity to grain silos, roads and dams/water bodies were assessed as risk factors for infection with HPAI H5N8. There was good agreement between the outbreaks and areas identified at higher risk of avian influenza. The recommendation was made for similar assessment of spatial risk factor for all the 2017 outbreaks in the northern provinces of South Africa. This can provide evidence towards understanding the ecology of HPAI in South Africa and inform management of HPAI.

Cumming, G. S., P. A. R. Hockey, L. W. Bruinzeel, and M. A. Du Plessis, 2008, Wild bird movements and avian influenza risk mapping in southern Africa. *Ecology and Society* **13**(2): 26. [online] URL: http://www. ecologyandsociety.org/vol13/iss2/art26/

A Single Vaccination with a Recombinant H5 Antigen Produced in Insect Cells Induced 100% Protection against an HPAI H5N8 Isolate from South Africa

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The evolution of highly pathogenic avian influenza viruses (HPAI) of an H5-lineage firstly described in 1996 in the Guangdong province resulted in HPAI encoding for several H5-antigenic clades and reassortment with different neuraminidase subtypes (H5Nx). The decended viruses of the H5-Guangdong lineage spread into several Asian countires first but in 2005 the European continent was reached. Subsequently the H5Nx viruses spread to Africa and North America. In 2017 sixty seven countries notified the OIE for the dectection of H5Nx (65) and H7Nx (2). This indicates that HPAI's of the H5Nx have the potential to become endemic in the wild bird population with a constant threat to spill over into domestic poultry. Biosecurity is the most important tool to prevent HPAI from infecting poultry. But vaccination of poultry associated with an exit strategy from vaccination is an important tool to fight ourbreaks of HPAI. To this end vaccines, which protect from clinical signs and prevent spread of HPAI in poultry settings by significant reduction of virus shedding, need to be available. In July 2017 an HPAI H5N8 of clade 2.3.4.4 was detected in South Africa. The virus spread in poultry flocks, wild birds, and was also detected in ostriches. The HPAI H5N8 is still present in the aforementioned species in 2018. In order to evaluate whether a commercial vaccine containing a baculovirus encoded recombinant H5 antigen can provide protection against this HPAI H5N8 lineage 2.3.4.4 observed in South Africa a vaccination and subsequent challenge experiment was performed in November 2017. The challenge virus was a representative HPAI H5N8 virus isolated from a wild bird in South Africa. SPF chikens were vaccinated once and 20 days after vaccination (pv) challenge infection was performed. Chickens were observed for 14 days after challenge infection (pc) and 100% protection from morbidity and mortality was observed. The nonvaccinated chickens died within 4 days pc indicating a valid challenge infection. A low level of viral RNA was detected by RT-qPCR up to seven days pc in oropharyngial swabs and up to four days in cloacal swabs. Furthermore, it was observed that the vaccine can be used in a DIVA (**D**ifferentiating **I**nfecting from **V**accinated **A**nimals) approach, based on the detection of antibodies against the nucleoprotein of influenza A viruses in the vaccinated and challenged chickens as early as seven days pc. The vaccine fulfilled all expectations of an inactivated vaccine after one vaccination against challenge with an emerging HPAI H5N8 virus and is suitable for a DIVA approach.

Analysing Production and Financial Data from Farmers to Identify Sustainable Poultry Enterprises in a Resource Constrained Rural Poultry Sub-Sector of Eastern Zambia

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There are limited data on production and financial performance of the rural poultry sector in developing countries like Zambia that could be used by extension services as a feedback loop to enhance service delivery in the sector. Thus, a study that used production and financial data obtained from poultry farmers of Eastern Zambia was conducted to describe the rural poultry sub-sector and conduct financial analysis. It compared the financial performance of indigenous chicken production to broiler and layer production. The aim of the study was to identify sustainable poultry enterprises in a resource constrained environment and identify knowledge gaps among poultry farmers. These could be used to initiate and enhance a participatory extension approach and build capacity of farmers in the sector. Descriptive, spatial, cash flow and breakeven analysis was used to analyse data obtained from 459 rural poultry farmers and expert opinion from 5 local extension workers.

Poultry ranked highest in terms of popularity and numbers when compared with other animals kept by respondents (median=20). Most poultry were kept under free-range and brood an average of 3.1 clutches. Except for annual set up costs, and some variable costs, the study could obtain data on most production costs and income generated from poultry farmers. Nevertheless, annual cash flow analysis conducted using costing data from poultry farmers and expert opinion of extension workers revealed that indigenous chicken enterprises had the highest net return on investment (0.17) compared to commercial broilers and layers with net returns of 0.03 and 0.06 respectively. Breakeven analysis revealed that indigenous chickens required the lowest number of products to be sold (18) to realise profit compared to broilers (1136) and layers (995).

The study identified indigenous chickens as the most sustainable production system in a resource constrained rural poultry sub-sector and discusses how these results can influence future resource allocation within the poultry industry.

Application of Spatial Scan Statistics and Spatial Empirical Bayesian Smoothing in Investigation of Spatial Patterns of Diseases

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Spatial patterns of disease occurrence can provide useful information on changes in disease patterns and identify communities at high risk of infection. This information can in turn be used to guide policy decisions on animal health and management of public health programs. Investigation of spatial patterns allows surveillance data with spatial references to be analysed and integrated as part of disease reporting. Descriptive maps are the first step in visualization and identification of possible spatial disease patterns. However, disease mapping in small areas suffers from the small number problem and therefore requires spatial smoothing that allows visual identification of spatial patterns while adjusting for varying sample sizes (non-homogeneous variances) across areas. One method of smoothing, Spatial Empirical Bayesian Smoothing (SEB), uses spatial weights to adjust for both non-homogeneity of variances associated with differences in sample sizes across areas as well as spatial autocorrelation (resulting from neighbouring areas having similar disease rates). The 2nd step in investigation of spatial patterns of disease is to assess for spatial autocorrelation in order to identify disease clustering at global and local levels. Some of the inferential statistical tests used to detect disease clusters include Kulldorff's spatial scan statistics, Moran's I, Cuzick-Edwards' and Tango's scan statistics. Kulldorff's spatial scan statistics can analyse discrete and continuous data using Bernoulli, Poisson, ordinal, exponential and normal distributions. We apply SEB and Kulldorff's spatial scan to clinical data of dogs presented at Onderstepoort academic hospital and investigate spatial patterns of *Staphylococcus* infections and antimicrobial resistance.

Using Prevalence Data To Estimate Seroconversion And Sero-Reversion Rates Of Rift Valley Fever Virus In An Endemic Area

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Rift Valley fever (RVF) is a mosquito-borne zoonotic disease causing large outbreaks amongst ruminant livestock in Africa, but recent studies suggest that RVF virus (RVFV) can circulate undetected in endemic areas. Force of infection and duration of seropositivity are key parameters in infectious disease epidemiology. This study used cross-sectional age-specific seroprevalence data to estimate seroconversion and sero-reversion rates in cattle, goats and wildlife in an endemic area, and to compare them with prospectively estimated seroconversion rates in the same area. Cross-sectional serological surveys using the serum neutralization test were conducted in cattle (n=606), goats (n=104) and wildlife (n=156) in far northern KwaZulu-Natal. Reversible catalytic models of the form: Pa = lambda/(lambda+rho)*(1−exp(−(lambda+rho)*a)), where Pa is seroprevalence in animals of age a, lambda is past seroconversion rate and rho is sero-reversion rate per animal-year, were fitted to seroprevalence data. Model estimates of past seroconversion rates were subjectively compared with current seroconversion rates calculated from follow-up of the seronegative livestock (and reported in a separate paper).

High seroprevalence in all species and age groups, even in young animals (25% in animals ≤2 years) indicated recent RVFV activity. Model estimates of the number of seroconversions per animal-year were 0.21 (95%CI: 0.11-0.39) for cattle, 0.18 (95%CI: 0.06-0.50) for goats, 0.33 (95%CI: 0.10-1.14) for wildlife and 0.26 (95%CI: 0.15-0.44) for all species combined. For livestock, this was 3 times lower than the 0.65 seroconversions per animal-year observed during the study period. This could possibly be explained by extremely low rainfall during previous years, followed by relatively normal rainfall during the study period. The combined crude estimate of rho suggested that average duration of seropositivity (1/rho) was 2.7 years (95%CI: 1.2-5.8). This study provided evidence of sustained RVFV circulation even during drought conditions. Reversible catalytic models provide a relatively simple way of estimating force of infection and duration of seropositivity using cross-sectional data and may be useful in epidemiological studies of RVF and other diseases.

Key words: Rift Valley fever, vector-borne diseases, force of infection, seroconversion, reversible catalytic model.

The 2015/2016 Trade Network of Live Equids Destined for Southern Africa

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UNCOMTRADE (https://comtrade.un.org) is a database that provides free detailed trade data between countries. It uses Harmonized System (HS) codes to categorise trade commodities. Included in these categories are the upstream HS code of 0101 which is the trade in live horses, asses, mules and hinnies. An analysis of all live equids traded during 2015 and 2016 was performed to establish the trade patterns of these animals in where their destination was one of the SADC (Southern African Development Community) countries.

Data from UNCOMTRADE comes either as a commodity count, weight or value of the commodity and data can come from both the country to where the commodity is traded to (the importer), or from the country of origin (the exporter). Data cleaning was thus imperative, and a final count of trade of equines between countries in this analysis was established first by the count; if count was missing by weight, with 400kg approximating one equine, and finally if weight data was missing then value was used as a proxy for count based on similar prior trade between the two countries.

Over the two year period 15 Southern African countries were a destination for equids from across the globe. Equids originated from 6 continents, with Africa accounting for 88% of the origin of these equids. There were 69 different trade routes (country to country) accounting for 7050 equids moved into Southern Africa from 34 different countries in the period reviewed. In terms of continental diversity: Europe (13 countries) and Africa (11 countries) had the most different countries involved in this trade. South Africa was the source of 75% of the total equids moved into Southern Africa, with Namibia (7%), Zimbabwe (4%) and Australia (3%) making up the majority of the remaining sources. Of the equids traded to Southern Africa, 12% come from outside Africa. 70% of these are destined for South Africa and 15% are destined for Tanzania, Seychelles and Mozambique, none of which exported to South Africa during the period reviewed. The South Africa – Namibia / Namibia – South Africa trade accounts for 19% of all moved equids into Southern Africa, a partnership where South Africa is the origin 66% of the time. The South Africa – Botswana / Botswana – South Africa trade accounts for 34% of all moved equids, however here South Africa is the origin 99% of the time.

South Africa can be considered a major source of equines for the Southern Africa region, as well as acting as a gateway for horses from outside Africa that enter the region. Having information about trade patterns should allow risk based trade decisions to be made, and this is particularly true for South Africa and its neighbouring countries and the future trade of equines. The is however room for an improved source of data given the extensive data cleaning that was required to establish a harmonised data set. No all countries report on the same HS level and this limits the global scale analysis that can be performed on these data.

A Freedom of Disease Survey: African Horse Sickness Virus in South Africa's Surveillance Zone in 2017

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An African horse sickness (AHS) virus (AHSV) outbreak occurred in South Africa's AHS controlled area in autumn 2016. A once off freedom of disease survey was performed to establish the likelihood of ongoing circulation of this virus during the same period the following year. The seasonality of AHS outbreaks, the authorized AHS vaccination period against AHS and the inability to differentiate infected from vaccinated animals on laboratory testing impacts the ability to perform a freedom of disease survey for AHS in the same year as outbreaks in the controlled area. During March 2017 a total of 336 randomly selected horses from 51 farms were sampled from the 2016 outbreak population at risk of 1817 horses. A base population level design prevalence of 1% was used to establish the sample frame and 3 within herd and herd level design prevalence scenarios were used in evaluating the outcome of the surveillance sensitivity and probability of freedom. Depending on the underlying design prevalence, effectively ranging between 0.8% and 6.4%, and the use of informed or uninformed priors the confidence of freedom from this surveillance ranged between 81.9% and 99.9% (uninformed prior) and between 97.9% and 99.9% (informed prior). Based on the results the authors conclude that it is unlikely that the 2016 AHSV was still circulating in the autumn of 2017 in the outbreak containment zone. The ability to perform freedom of disease surveys, and also to include risk-based surveillance, in the AHS controlled area of South Africa is influenced by the changing underlying population at risk and the high level of vaccination coverage in the horse population, and ongoing census post-outbreak must be undertaken to maintain a representative sampling frame for future surveillance activity.

Middelburg and Sindbis Alphaviruses as a Cause of Febrile and Neurologic Disease in Horses and Humans in South Africa

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Alphaviruses belong to the family Togaviridae and include known pathogens such as Chikungunya -and Semliki Forest-virus that cause arthritic disease and encephalitis in humans and animals globally. The Center for Viral Zoonoses (CVZ), University of Pretoria (UP) has previously reported two of these alphaviruses, Middelburg- (MIDV) and Sindbis-virus (SINV) as the etiological agent responsible for neurologic disease in horses in South Africa. Horses may serve as an early indicator of circulating arboviruses with disease causing potential.

Clinical samples, including EDTA blood, serum and organs of horses with acute or fatal neurologic and febrile infections were sent to the CVZ, through an established surveillance program by veterinarians across South Africa. Specimens selected from 2014-2017 were screened by real-time PCR for Alphavirus, Orthobunyavirus and Flavivirus genera (IgM ELISA for the Flavivirus, West Nile Virus (WNV) was also performed). Human CSF specimens, were sampled between February to May 2017 from patients with unexplained neurological symptoms submitted to the National Health Laboratory Service, Tshwane Medical Virology laboratory, UP and PCR screened for alphaviruses only. These specimens are from hospitals and clinics across the greater Gauteng region. Two veterinary students whom displayed neurological and fever signs during this period were also included in this study.

Between 2014 and 2017 a total of 982 horse specimens were received. The majority 683/982 (69.5%) had neurologic signs and 222/982 (22.1%) fever, the remaining 77/982 (7.8%) cases displayed signs such as abortion, icterus, nasal discharge, orbital swelling and pneumonia. Most of the specimens were submitted from Gauteng (39%, 383/982), followed by the Western Cape (19.34%, 190/982) and KwaZulu-Natal (11.4%, 112/982).

In total 64/982 (6.5%) tested positive for MIDV and 10/982 (1%) for SINV. For MIDV positive cases 31.3% (20/64) presented with neurologic signs and fever, 30% (19/64) with neurologic signs only, 32.8% (21/64) with fever, 1 was an aborted fetus and 1 had signs of icterus. For the 10 SINV positive cases 5 had neurologic signs, 4 fever and 1 pneumonia. Numerous MIDV cases were detected in 2017 (10.1%, 44/437) specifically from Benoni and Bapsfontein (31.8%, 14/44) in Gauteng. Of the 44 MIDV cases 4 had neurologic signs only, 20 fever and neurologic signs and 16 only fever. From 2014-2017 there were 6 fatal MIDV positive cases ,5 had neurologic signs and 1 was an aborted

fetus. Four of these six were MIDV/WNV co-infections. For SINV positive cases there were 2 fatal cases, 1 with fever and 1 with neurologic signs. Of the189 human CSF specimens screened, 3 MIDV positives were detected in a 49-year-old male, a 30-year-old female and a 2-year-old boy, all whom presented with unexplained neurological symptoms. Of the 2 veterinary students, 1 tested positive for MIDV. She presented with neurological signs following the MIDV infection but made a full recovery.

The ongoing surveillance program has demonstrated that MIDV and SINV are circulating annually in South Africa and are a cause of morbidity and mortality in horses and humans and highlights

Taylorella SPP. in South Africa: The Saga Continues

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In May 2011, the first South African case of *Taylorella equigenitalis*, the causative agent of contagious equine metritis (CEM), was confirmed on samples from an index property in Gauteng [1]. The combination of a traceback of in-contact horses and their offspring and the institution of a nationwide stallion screening programme using qPCR identified a total of 39 positive horses (36 stallions & 3 mares) with a subpopulation focus at the South African Lipizzaner Centre in Gauteng.[2]. On bacteriology, isolates were obtained from 23 of these stallions and 2 of these mares. All confirmed cases were successfully treated and subsequently tested negative on multiple follow-up tests [3]. Newly-introduced national legislation currently requires all breeding stallions to be tested for *T. equigenitalis* every 2 years to obtain a CEM clearance certificate [4].

Multilocus Strain Typing (MLST) on crude extract obtained from positive cases from the South African outbreak identified only one strain type, designated ST-4 [5, 6], previously only identified in France and Austria and linked with the Lipizzaner breed [6].

In 2015, due to increased awareness of CEM, semen straws collected and frozen from a stallion at the index property in 2008, prior to outbreak identification, were submitted by a veterinarian for testing prior to use. The semen tested positive for *T. equigenitalis* on qPCR. Subsequent follow-up testing of the semen donor (now a gelding), after genital swabbing, was positive for *T. equigenitalis* on both qPCR and bacteriology[4].

In 2016, a CEM certificate requested from a stallion not previously used for breeding was positive on screening and this was subsequently confirmed. Traceback of all horses on the property yielded an additional positive stallion. Both stallions were quarantined and successfully treated. Epidemiological back tracing into this outbreak is still ongoing.

Also in 2016, post-quarantine testing of 2 imported miniature donkeys was positive for *Taylorella asinigenitalis,* the first recorded incursion of this organism into South Africa. *T. asinigenitalis* is considered non-pathogenic in horses, but does cause clinical signs of

endometritis under research conditions [7]. Both donkeys were treated successfully under quarantine conditions.

In conclusion, ongoing vigilance both via national surveillance and during post-entry quarantine is required to prevent further incursions or the establishment of *Taylorella spp.* in South Africa which may result in considerable economic losses to the South African horse industry and associated interference international trade and movement of horses.

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Failure to Detect Equid Alphaherpesvirus 1 DNA in Thoroughbred Placentae and Healthy New-Born Foals

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Equid alphaherpesvirus 1, more commonly known as equid herpesvirus 1 (EHV-1), is a respiratory virus that has been associated with poor performance in racehorses as well as late term abortion, neonatal foal death and encephalomyelopathy in other populations. Horizontal transmission of the alphaherpesviruses is well defined, however

evidence of vertical transmission of EHV-1 or -4 in association with the birth of a healthy foal has not been described and would inform the refinement of strategies for prevention and disease control measures on stud farms. The aim of the study was to sample a cohort of mares, their healthy neonates and the foetal membranes to test for the presence of both EHV-1 and EHV-4 using a quantitative polymerase chain reaction (qPCR) assay (Diallo et al., 2006). Maiden and multiparous Thoroughbred mares (n=71) aged between 5 and 19 years of age, resident on a single farm in the Western Cape Province were included in the study. Pregnant mares were routinely vaccinated for EHV-1; no recent outbreak of EHV-1 associated disease had been reported. Fetal membranes were sampled immediately following post-partum expulsion, using cotton-tipped sterile swabs for viral nucleic acid detection via qPCR. Venous blood samples and nasal swabs were obtained from both mare and foal 8 h post-partum for qPCR assay to detect the presence of viraemia or viral shedding, respectively. All swabs were stored at 5 °C until transport via courier at ambient temperature to the Veterinary Genetics Laboratory, University of Pretoria for testing. Neither EHV-1 nor EHV-4 nucleic acid was detected. In conclusion there was no active shedding of EHV-1 and EHV-4 at the time of sampling and thus there was no evidence for vertical transmission of these viruses on this stud farm during the sampling period.

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Socioeconomic Impacts of Working Horses in Urban and Peri-Urban Areas of the Cape Flats, Cape Town, South Africa

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In the Cape Flats townships, Cape Town, South Africa, there are in excess of 250 working horses, known as 'cart horses' in the community. They serve the community with scrap metal collection and transportation, garden refuse removal, human transport and the general selling of goods. A questionnaire was undertaken to understand the social and economic impacts the use of a horse and cart in the Cape Flats has on individual owners and/or drivers, their households and the surrounding community. Welfare impacts on the horse itself were also considered. An understanding was also obtained on the spatial extent to which the cart horses work and how that relates back to the impact on the horse and participant of the survey. A mixture of classical quantitative questions combined with qualitative participatory technique questions were used to gain a well-rounded response.

A total of 100 participants took part in the questionnaire, who worked with 163 horses between them. The majority (89%) identified the working horse income as their primary source of income. Over and above the participants, an additional 716 other people were supported financially through income generated by the working horses, where the mean number of children supported was 2.9 (95% CI: ± 0.42) per interviewed participant. Scrap metal transportation was the most common function of the working horse and season (winter), pregnancy and shoeing were important factors negatively impacting on their ability to work.

It was demonstrated that the cart horse industry was impactful on not only those who worked in the industry, but also the surrounding residents either through their work or through supporting others with their income. This study reveals the concepts of 'One Health' and 'Health in Social-Ecological Systems' in action as horse and human health and welfare within the Cape Flats are so closely intertwined.

Link Between *Listeria Monocytogenes* and Processed Meats

Elna Buys

The Role of Education, Prevention and End Point Testing in Detecting and Controlling Listera Monocytogenes

Elna Buys

The Listeriosis Outbreak in South Africa: Useful Microbiological Testing for Listeria Monocytogenes

Lucia Anelich

Consumer Rights and Civil Class Action Cases involving Listeria, and the Role this could Possibly Play in the South African Response to *Listeria Monocytogenes* in Processed Food

Januz Luterek

Abstract

The Listeria outbreak has brought the topic of the legal obligations of producers and retailers and the liability, both criminal and civil, front and centre. It is unfortunate that the Department of Health did not deem it necessary to include Listeria and limits for Listeria (whether L.monocytogenes or any other) into the Microbiological Standard Regulations R 692 although the Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972 has a general prohibition on the manufacture and sale of contaminated foodstuffs. Failure to comply with the FCD Act can lead to criminal prosecution after inspection by an EHP. In addition, consumers can have a claim for any harm caused or even bring a class action law suit in the name of all consumers who suffered harm – this is in terms of the Consumer Protection Act.

Persistence of Different Listeria Monocytogenes Strains from Processing Plants

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Abstract

Molecular subtyping of *Listeria monocytogenes* isolates associated with listeriosis outbreaks has invariably shown that contamination of finished products arises from strains resident in food processing plants and that such strains cause recurrent product contamination from the same plant over a long period time. The underlying reasons of this persistence are yet to be fully elucidated. This paper highlights the aspects of the ecological basis of *L. monocytogenes* persistence, supported by data from recent studies conducted in our laboratory at the University of Pretoria. The paper also brings to the fore, the roles of stress responses and biofilm formation as factors that aid *L. monocytogenes* persistence.

Keywords: *Listeria monocytogenes*; persistence; persister cell

Establishing Microbiological Risk Management Frameworks in Meat and Meat Products

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Abstract

Food safety risk management can be described as the process of weighing control alternatives taking into account scientific information on risks to consumers as well as other relevant inputs (economics, technical feasibility, societal preferences) and choosing and implementing food safety measures as appropriate. Food Safety Objective (FSO) concept translates public health risk into a definable goal: a specified maximum frequency and/or concentration of a hazard in a food at the time of consumption, which is deemed to provide an appropriate level of health protection. Maximum hazard levels at other points along the food chain are called Performance Objectives (POs).

There is no legal obligation for national government authorities to adopt ISO and or Codex Food Safety Objective (FSO) into domestic law, however, if national government authorities are to set a FSO that is more stringent than the relevant internationally agreed standard obtained through the Codex process, they would need to have clear justification, based on public health considerations with respect to food safety and sound scientific evidence. The approach of FSO and PO enables the food industry to meet a specific target by the application of the principles of Good Hygienic Practice (GHP), Hazard Analysis Critical Control Point (HACCP) systems, performance criteria, process/product criteria and/or acceptance criteria.

In South Africa, the FSO and PO are set in the Meat Safety Act 40 of 2000 where sections 51 & 52 of Part III to the poultry regulations (No.R.153) requires application of a risk-based approach to hazard identification in poultry meat and a subsequent development of hygiene management programs to prevent, eliminate or reduce the identified potential hazards in poultry. Though the regulation does not clearly distinguish a hygiene management program from a hazard management program by definition, there is an assumption that the plant's hygiene management programs should address all hazards classified as potential threats (intentional and unintentional hazards). The establishment is expected to support any decision that is made during its hazard analysis program. Above all, the hygiene management program of the establishment must contain verification procedures that ensure the system is working as designed. Where critical limits are not met, appropriate corrective actions must be instituted before the product is released on the market. The Appropriate Level of Protection (ALOP) set in

the regulations and procedural notices are deemed sufficient to protect the consumer from potential food-borne hazards.

Listeria Hysteria
Public Perceptions and Misconceptions

Christo Labuschagne

The current Listeriosis outbreak that South Africa is facing has been documented as the world's biggest outbreak in history.

A bit of background: In 1911 the bacteria was isolated from diseased livers of rabbits. Over the years the bacteria has had a couple of name changes but in 1940 it was named *Listeria monocytogenes* as it is known today after Joseph Lister, the British surgeon who discovered sterilizing surgical instruments before operations reduced the risk of post-op infections.

According to the United States Food and Drug Administrations (FDA) – "*Listeria monocytogenes* is rare but serious".

Some statistics at a glance:

- The World Health Organization (WHO) estimated that in 2010, 23 150 cases of listeria was reported. Of this a total of 5 463 deaths occurred.
- In the United States reported cases are between 1 600 – 2 500 with 260 – 500 deaths annually
- In Europe a total of 1 645 cases and 270 death occurred in 2009

The United States estimates that the total annual cost because of Listeriosis is around 2.6 Billion dollars making it the third most costly foodborne illness in America after Salmonellosis and Toxoplasmosis. The main areas that make up these costs are:

- Medical expenses
- Decreased productivity
- Premature death

So what is Listeria? Characteristics

It is a zoonotic BACTERIA that is widespread in the environment (soil and water). Animals, particularly cattle, carry *Listeria monocytogenes* without appearing sick and shed the bacteria in their faeces (illegal slaughtering…). The bacteria can tolerate both acidic and salty conditions, both high and low temperatures and fairly low moisture content environments. This is what makes listeria so difficult to control and results in intermittent contamination of food.

At low temperatures, *Listeria monocytogenes* can be a potential problem in properly refrigerated food and even in frozen raw products. This bacteria thrive in cold environments.

People become infected by eating and handling contaminated food. By touching contaminated surfaces and utensils and then accidentally transferring the bacteria from your hands to mouth

can also result in infection. Babies can become infected in utero or at birth if their mothers ate contaminated food during pregnancy.

The bacteria can contaminate a variety of food:

- Raw meat
- Ready-to-eat processed meat (hot dogs, deli meats etc.)
- Raw vegetables
- Refrigerated Pates
- Ready-to-eat smoked seafood and raw seafood
- Prepared or stored salads
- Soft cheeses
- Unpasteurized milk and milk products

Pasteurization, cooking and most disinfecting agents kill *Listeria monocytogenes*.

Listeriosis in People

The bacteria is found in almost exclusively pregnant woman, new-borns and people suffering from a weakened immune system (cancer patients and other diseases).

One gets infected by ingesting the bacteria which then grows and multiplies in the liver and is spread all over the body via the bloodstream and manifesting in the brain (encephalitis), causing meningitis and gastrointestinal conditions such as vomiting and diarrhoea. The incubation period varies between 24 hours to 60 days.

Most common symptoms:

- Vomiting
- Diarrhoea
- Fever (flu-like)
- Fatigue
- Muscle aches

Listeriosis in Animals

The bacteria can infect many animal species. Dogs and cats rarely get listeriosis (usually do not show signs). It is mainly a disease associated with ruminants (cattle, goats, sheep) with sheep being particularly sensitive. These animals can become infected by eating spoiled silage. Common signs include: encephalitis with neurological signs like loss of balance, circling and unusual spasms.

Other clinical signs include:

- Fever, loss of appetite and decreased activity
- Some cases can have late term abortions

With regards to companion animals, rabbits and rodents are more commonly infected by *Listeria monocytogenes*. Some signs include:

- Septicaemia
- Uterine infections

- Diarrhoea or constipation

Dogs can be infected with Listeria by the same sources as human exposure (Infected meats and dairy products and other contaminated foods). Dogs should not be fed products that were destined for re-call. There have been 6 reported cases in dogs from 1947 – 2000 with a wide range of signs. The ever increasing popular trend of feeding raw diets also carry a higher risk if the food is contaminated. As mentioned before, healthy dogs rarely get infected. It is mostly young puppies and older dogs that are more likely affected.

Clinical signs are non-specific

- Diarrhoea
- Nausea and vomiting
- Weakness
- Fever
- Muscle sores
- Lethargy
- Stiff neck
- Lack of coordination and circling
- Skin infections
- Facial nerve paralysis
- Depression

In cats the clinical signs depend on an individual response as not all cats develop *Listeria monocytogenes*.

How to diagnose:

- A complete physical examination
- Take a complete history
- Review the animals's medical record
- Ask diet related questions (raw meat diet, dairy)
- Blood smear (monocytosis)
- G(+) short rods (singly pairs, chains)
- Facultative intracellular pathogen – in macrophages/epithelial cells
- CSF (in neurological cases)
- Urinary and faecal samples
- Any other test to eliminate other causes

Diagnosis is typically done with blood tests (and in neurological cases cerebral spinal fluid can also be used) to run bacteriology tests on the samples.

Treatment (symptomatic)

- Antibiotic treatment: Trimethoprim-Sulfamethoxazole, Amoxicillin, Penicillin, Ampicillin (Fluoroquinolones)
- Severe cases hospitalized with a drip (fluids) and pain medication

The prognosis depends on the animals overall health as it can be fatal.

Because it is a zoonotic disease, dogs can spread *Listeria monocytogenes* to people – According to Ontario Veterinary Medical Association. It is highly unlikely that a dog that has eaten

contaminated meat would pass the infection on to humans, but it is possible. A small percentage of healthy pets can shed Listeria in their faeces at any given time.

The South African context

Apart from the health threat that Listeria holds, it also has major economic consequences on the meat industry. It has been estimated that the Pork Industry is losing around R45 million/week and many jobs in this industry are at risk. According to Johan Kotze (CEO SAPPO) the industry is in a crisis. "Listeriosis is effecting every farmer involved and it is a massive challenge".

Normally the demand for pork meat is about 50% for fresh products and the other 50% for processed products. There has been a huge decrease in the demand for processed products – a decease by up to 75%. Estimated processed meat losses are around R800 million/month. Now all of a sudden you don't sell processed products so consumers go back to fresh products. What does this mean? The biggest impact has probably been on small scale farmers. Those that have been affected run the risk of bankruptcy and job losses. This all because of a decrease in the price of their produce (40-50%). Ultimately as less farmers are able to survive, the market will have a supply shortage and higher meat prices will occur.

Registered pig farmers with SAPPO: 125 commercial farmers and 400 upcoming/emerging farmers. So it is clear that the effect of such outbreaks have a much bigger effect on the majority of farmers in the country.

The source of the Listeria outbreak: Enterprise Foods Polokwane Factory

The Head of the Centre for Enteric Diseases at the National Institute for Communicable Diseases (NICD) Dr. Juno Thomas said that these bacteria should not be in any food factory as contamination can easily occur with Listeriosis. It was mentioned that the Polokwane factory has a serious problem with food safety. On the 2nd of February over 300 samples were taken. Sixteen of these samples came back positive for ST6-Type strain which caused 91% of cases. Around 100 of the samples came back positive for other strains of *Listeria monocytogenes* which can cause food borne illness.

Fortunately there is no need to panic according to Dr. Kerriga McCarthy, Head of the outbreak response NICD. "*Listeria monocytogenes* affects people with a weakened immune system (immune-compromised individuals); the elderly; pregnant woman and babies or infants".

The vast majority of people who consume these products will be fine and there is no need to worry. "In the absence of symptoms one should not worry. If someone that falls in the above category and that has consumed these contaminated foods developed stomach cramps, diarrhoea or fever – go to the doctor and get tested".

Listeria monocytogenes loves your fridge. It can continue to multiply in uncooked food kept in the fridge. This is why processed meats, smoked meats and soft cheeses that are not cooked are often linked to outbreaks. The NICD recommended the use of diluted bleach to clean areas where you may have kept processed meats.

Cooking kills Listeria so it is advisable to properly cook food before consumption. Food should be heated above 70 degrees Celsius. Keep cooked food separate from raw food and utensils used on raw food must not touch cooked food. This is to prevent and avoid cross contamination.

Listeria can be difficult to find. The bacteria is not spread homogenously through food so scientist can test one slice of polony and find nothing then test another slice and find the bacteria.

What are the ways forward?

- Negotiations between government and the relevant role players to devise a strategy and protocols to
- Learn from the outbreak to prevent it from happening again
- The lost trust from consumers: rebuild the pork industry and regain trust of a brand
- One live lost is one too many

Risk-Based Approaches to Managing Food Safety in South Africa: A Scientific Review with Reference to Listeriosis outbreak in South Africa

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Abstract

Compliance with local regulations and international standards is an essential part in the food industry to ensure food safety and business growth. This presents challenges for those in charge of a food processing operation, especially if personnel are not adequately trained. Government authorities and Industry have a joined role to play in ensuring the safety of food that is consumed by the public. The on-going Listeriosis outbreak, in South Africa, the largest recorded in the world, that has resulted in about 183 deaths and 948 confirmed clinical cases, (http://www.nicd.ac.za/wp-content/uploads/2018/03/Listeria-Sitrep-03Mar2018.pdf) has put risk-based approaches to food safety prevention and control in the spotlight. This has shown that the impact of microbiological contamination in products can be potentially huge, particularly in terms of risk to public health, if food safety control measures are not strictly adhered to.

The implementation of effective food safety management systems (FSMS) using Hazard Analysis and Critical Control Point (HACCP) system integrated with the contemporary approaches to risk management of food supply chain threats on raw materials, product integrity and authenticity based on Food Defense system (TACCP - Threat Assessment Critical Control Point) and Food Fraud (VACCP - Vulnerability Assessment Critical Control Point) has become very pertinent. While Government authorities have the responsibility of monitoring and ensuring compliance in the control of food safety in-line with national regulations, industry equally has the responsibility of complying with their certified FSMSs. These FSMSs that include the new BRC Global Standard for Food Safety issue 7 with additional food security features and the Global Food Safety Initiative (GFSI) based FSSC 22000 system, require food manufactures to satisfy the requirements of risk-based food safety prevention and control. Government authorities and Food Manufacturers need to have an in-depth understanding of using HACCP, TACCP and VACCP tools, and have a sufficient competencies to be able to effectively control and prevent food hazards in the food value chain.

This paper reviews the role that government authorities and food manufacturers each play in ensuring production of safe food for human consumption in South Africa. Gaps in the South African food legislation and weaknesses in the food industry that have led to the current unprecedented Listeriosis outbreak are reviewed. Recommendations on actioned that both industry and government must do to prevent and control any future foodborne disease outbreak are given.

The Role of Envorinmental Health Practitioners in the South African Listeria Monocytes Outbreak Detection and Response

Rina Nel

Die rol van ‘n Omgewingsgesondheidspraktisyn(OGP) gedurende ‘n uitbraak van Listeriose. Die wetgewing waaronder ‘n Ongewingsgesondheidspraktisyn aangestel word, asook die magte van die OGP. Die betrokkenheid van OGP’s by die “Incident Management Team” (IMT) as deel van die “National Institute for Communicable Diseases” (NICD) en ‘n Opsomming van die aktiwiteite van hierdie span gedurende die uitbraak in Suid-Afrika.

The Role of National Institute for Communicable Diseases in South Africa

Kerrigan McCarthy

The mission statement of the National Institute for Communicable Diseases' states that the organisation will be a resource of knowledge and expertise in regionally relevant communicable diseases to the South African Government, to SADC countries and the African continent, in order to assist in the planning of policies and programmes and to support appropriate responses to communicable disease problems and issues. To that end, the NICD conducts surveillance for communicable diseases and supports outbreak investigations for diseases of public health importance. The NICD has a number of surveillance programmes including national notifiable medical conditions (NMC) surveillance, the GERMS-SA laboratory-based and enhanced surveillance programes, and numerous sentinel site surveillance programmes for clinical syndromes including diarrhoeal and respiratory diseases and sexually transmitted infections. At the time of the listeria outbreak in mid 2017, listeriosis was not notifiable. A spike in cases was reported to the NICD by clinicians from CHBaragwanath hospital, and by clinical microbiologist from Tshwane academic hospital. Subsequent review of NHLS and private laboratory information systems revealed a 10-fold increase in cases over the 2016-2017 period. Subsequently, listeriosis has become notifiable, and is reported through the NMC surveillance system. Refinements to the surveillance programme, including revisions of the case investigation form are underway. The presentation will review the process of the listeriosis outbreak investigation and how the source was determined

Gauteng Department of Health: Repose to the Listeriosis outbreak.

Mary Madaure

1. Introduction

Listeriosis in South Africa was not a notifiable medical condition until December 05, 2017. The minister Dr A. Motsoaledi declared it notifiable after a gradual increase in the number of laboratory confirmed cases were detected, investigated and confirmed by the National Institute for Communicable Diseases (NICD) around October 2017.

Most cases were detected at Steve Biko Academic Hospital (SBAH) around August 2017 and Chris Hani Academic Hospital (CHBAH) July 2017.

Gauteng is the smallest province in the country with an estimated population of 13.64 million. It occupies 1.5% of the land range and is administered under 5 regions (3 metros and 2 district municipalities).

2. Background and discussion

Listeriosis is a food borne disease caused by the bacteria pathogen Listeriosis Monocytogenes. The common symptoms include fever and flu like illness, nausea, vomiting, diarrhea, general body aches and pains. The population at risk includes pregnant women, neonates, the elderly and immunocompromised individuals such as persons living with HIV/AIDS, Cancer, Diabetics, Chronic liver and kidney diseases. The common strain causing the outbreak in SA has been confirmed to be ST6 by NICD. The outbreak is regarded as the largest to have occurred globally and is associated with contamination of ready to eat foods traced to an Enterprise Factory in Polokwane.

3. PROBLEM FORMULATION

As at 21 May, a total of 606 cases have been confirmed in the province with 106 deaths. For this current year of 2018 alone, a total of 172 new Listeriosis cases have been laboratory confirmed and reported in the province with 15 related deaths. With the nationwide total of 10334 cases, Gauteng bears the brunt (59%) of these cases.

4. RESPONSE TO THE OUTBREAK

4.1 Provincial Outbreak Response Team Activation

The province has an active outbreak response team which meet on monthly basis and as a need arise. Towards the end of 0ctober province was advised by National Department of Health (NDOH) to work closely with National Institute for Communicable Diseases (NICD) on addressing the suspected outbreak as most cases were detected within Gauteng.

Meetings and trainings were held, starting November 2017 to sensitize the teams and health professional on the disease (Listeriosis) and the expected outputs from different role players to curb the outbreak. this meetings and trainings were started at provincial level and cascaded down to the districts and sub-district outbreak response teams who in turn cascaded down to health facility level.

Over and above the response teams, we have six whats' up chat groups (1 provincial and 5 district) where urgent information is communicated.

4.2 OBJECTIVES

- to determine the point source of the outbreak

- to determine the magnitude of the problem and
- to implement control and preventive measures.

5.3 RESPONSES BY DIFFERENT STAKEHOLDERS

5.3.1 CDC/Surveillance and IPC (Hospitals

Surveillance of cases was heightened in all health facilities

- to determine the number and outcomes of all cases starting January 2017
- to ensure that case investigation forms were filled in and submitted to NICD and
- monitor case notification according to the requirements of Regulations relating to the surveillance and the control of notifiable medical conditions (R1434 0F 15 December 2017)

5.3.2 Environmental Health

Environmental health key focus areas during the outbreak

a) Epidemiological investigation at household level
    - Food history investigations at house hold level of notified cases with the aim of establishing food preferences including common source and food hygiene practices. Not all cases were investigated/followed up due to the following challenges
        - lack/ insufficient addresses
        - outcome of some patients not known
        - some families not cooperating due to the loss of their loved ones
        - some cases simply not notified especially even though the disease was declared a notifiable condition(lab confirmed information only)
        - some cases residing outside the provincial borders of Gauteng.

    - Collection of foods from refrigerators for LM testing. All food products testing positive for LM traced back to the supplier for further investigations, hence the detection of the point source of the outbreak.
    - Awareness on Listeriosis and promotion of five keys to safer foods practices is also done.

b) Food and environmental investigations at food premises

    This was the most exciting part as it presented some challenges and gaps in the implementation of food control legislation.

    - Food and environmental samples were collected at production sites, packaging and distribution points and retailers to screen for LM.

Positive samples subjected to serotype sequencing (WGS) a time consuming activity. Food samples ranged from raw (poultry, red meat, vegetable and fruits) to ready to eat foods(processes meat products, salads dairy)

NB!! Weekly reports were submitted from each municipality.

- Inspection of the food premises with emphasis on the structural conditions and operational hygiene practices where LM was isolated. PROHIBITION NOTICES were issued in certain instances where none compliance was found.(risk base approach)

c) Monitoring of the recalled products

With the announcement of the point source of the outbreak, implicated foods were recalled by the National Consumer Council (NCC). The role of EH was to monitor the process to ensure that, all implicated products are removed from the shelves and properly disposed of (incinerated) as dictated to the industries by the National Consumer Council.

Monitoring is successfully implemented and the process still continues especially with the destruction of the implicated foods. Gauteng is hosting the national storage of the industry with tons of products. Of the 4 incineration sites outsourced by industry only two are within the province.

5.3.3 Risk communication (Health Promotion and EH)

District and Local authorities were requested to develop Risk Communication plans. This were done and implemented with success through Health Promotion, Environmental Health, CDC/surveillance and Communications units

- trainings on Listeriosis and promotion of five keys to safer foods were done
- community at large, food handlers, risk population (health care facilities , ECD's, old age homes etc.) were targeted
- the electronic and print media were also utilized
- some districts developed their own IEC material
- media statements issued at municipal and provincial level until advised to leave that to the Minister of Health

City of Johannesburg is commended in this regard followed by Westrand District Municipality.

5.3.4 Gauteng Listeriosis Outbreak Joint Operations Committee

Food control and legislation is a mandatory activity shared by different departments in the country. During this period food industries were inundated

with visit from different spheres of government all with the same objective. This committee was set up between GDOH and GDARD with alternate chairing role between the two departments. The objectives of the committee was to;

- clarify and harmonize the different mandates (Environmental Health (EH) and Veterinary Public Health(VPH) in food premises particularly in abattoirs and Z83 processing plants
- plan for joint operations to ensure maximization of resources and
- reduce over regulation of food industries in desperate times

5.3.5 Political support

Our principals at all levels were updated throughout the process through reports and presentations. They in turn provided resources where necessary and supported any remedial actions taken to safe guard the health and wellbeing of our communities.

6 OUTCOMES

1. Approximately 50% all the cases are accounted for.
2. The point source of the outbreak was identified through trace back of foods that tested positive for LM in this province.
3. Some of the challenges and gaps in implementing food control and legislation identified are currently being addressed by the NDOH through the Integrated Multisector Team coordinated at NICD and assistance from WHO.
4. Coordination and collaboration with other role players within the government sphere proved to be crucial in implementing corrective measures whilst maintaining public confidence. This are some of the highlights
    - a workshop was conducted where the different roles and mandate were outlined
    - training of VPHO's and EHP's on sampling and risk base approach in assessing food processing plants were conducted
    - sharing of sampling results and any another relevant information
5. Political commitment in supporting and strengthening system geared towards ensuring public health safety.

7 CONCLUSION

The response to the outbreak tested the readiness and effectiveness of the provincial outbreak response team and systems in the control and prevention of food born outbreaks. There were lessons learnt along the response but in turn, presented opportunities for improvement.

8 ACKNOWLEDGEMENTS

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Environmental Health , Municipal Health Services; City of Ekurhuleni, City of Johannesburg, City of Tshwane; Sedibeng District Municipality and Westrand District Municipality - Department Health and Social Development,- Gauteng province,
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National Institute for Communicable Diseases – National Health Laboratory Service,
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Scrutinizing the Spread of Listeria

Listeria in the 21st century -Behavior in food processing and food distribution in the “global village”

Georgina Pondayi

Hazard Analysis at Critical Control Points has been introduced in food industry to improve control of Listeria and other foodborne pathogens. RTE foods is associated with sporadic illnesses or outbreaks of listeriosis (CAC, 2009). Cooking or pasteurizing all foods is believed to eliminate the risk of foodborne listeriosis entirely, but however we should take into consideration that modern food preferences emphasize the “wholesomeness” of raw and minimally processed foods as part of a normal diet. It is therefore crucial to sensitize the public that consumption of raw and uncooked food items is a risk to health.

Based upon current understanding of listeriosis outbreak in SA and public health impact, changes are needed to the current approaches or focus on the control of L. Monocytogenes. Listeriosis is almost always caused by exposure to a food source that was contaminated somewhere along the food chain, and raw materials that enter processing facilities carrying

listeria are major sources of contamination. Storage conditions must be adequate both at the food production level and in retail establishments, to avoid a high level of growth of this pathogen because cross-contamination is a major factor for the introduction of L. monocytogenes to foods. We must go beyond traditional training, testing, and inspectional approaches to managing and controlling listeria outbreaks.

There has been a lot of talk on factors predisposing to or sustaining persistent plant contamination by Listeria and opportunities for growth of the pathogen. The potential for Listeria to grow in a particular food during storage and distribution has been a key factor in determining the level of consumer risk and has been the basis of risk categorization by some regulatory authorities (Farber et al., 2011) and associated microbiological criteria (European Commission, 2005).Although some exports markets have a zero tolerance to L.mono in products even those that will be cooked ,several authors have concluded that it is virtually impossible to permanently eradicate L. monocytogenes from food environments because of its ubiquitous presence in the environment and many potential avenues for entry into the facility. How then can the entire food chain work together towards controlling outbreaks: collaborative efforts and sharing ideas via SQA, adequate hygienic design of a food premise and equipment, effective cleaning and sanitation, personnel practices and movement of people and materials into areas where food products are exposed. Along the food chain, good food-handling practices can significantly reduce the risk of contamination. 'Reshaping' manufacturing plants to reduce risk of Listeria is important .

One area that is not much published and discussed is the human dimension of food safety. Human behavior is often referred to as the “soft stuff”. In one facility contract workers were noted lying on the grass, eating from street vendors and littering the whole area waiting to start their shift. No sanitary facilities were noted in sight. When time was up they clocked in and a question was raised as to why the staff sleep on the grass during the day. They lived too far from the factory and thus preferred not to go home for the next shift. We require a better understanding of the human dimensions of food safety. If staff are not careful and do not take proper care in removing their soiled garments and washing their hands with hot soapy water after sleeping on grasses, touching drains, etc. they could potentially spread Listeria or other bacteria from one area to another, as well as onto food.

In another scenario three quarters of employees are contract workers with very high staff turnover. If plant managers know how Listeria contaminates food then they can pass this information to all their staff. With the proper training and resources staff can identify Listeria problem areas, reduce the risk of listeriosis with proper cleaners and cleaning practices, and keep their work environment controlled against future outbreaks. Food safety must be treated as a non-competitive issue and embedding sustainable food safety behaviors into the existing company culture will lead to better control measures for listeria. Although most of raw food is normally eaten after cooking, the risk of listeriosis should not be underestimated, as the consumption of raw foods such as sushi, and undercooked hamburger is a reality.

Listeria in Gauteng Province

Yemi Akerele & Shepherd Kamudyaiwa

Listeriosis has always occurred at typically low incidence in the South African population. However, 2017 saw an uncharacteristic upsurge in the number of cases as well as fatalities in humans. This rapid spread and the unusual/unexpected shift from the normal behaviour in the disease resulted in the disease being classified as a Category 1 Notifiable Disease by the Minister of Health on the 5th of December 2017. This change in classification appeared in Government Gazette No. 41330 of 15 December 2017.

At the time of the notice from the Ministry of Health the confirmed distribution of cases in humans were as follows:
Gauteng had 62% (345/557; Western Cape had 13% (71/557); KwaZulu Natal 7% (37/557);
66% of the total cases were in the public sector whilst 34% had been recorded in the private sector(1). As of May 2018, 10?? Cases had been diagnosed. By the 31st of December 2017 the media reported to have found the source of Listeria in an abattoir in Gauteng which got GDARD up on our feet working tirelessly to consider confirm or refute the allegations.

All active abattoirs were sampled for **Listeria spp** with interest in **L. Monocytogenes** of which by the end of the first-round results indicated n=57, 56% negative and 44% positive. The positive abattoirs were subjected to a cleaning and disinfection regime and by the end of the second round of sampling n= 21, 81% negative and 19% still positive.

Isolates (n= 53) from the abattoirs are yet to be serotyped to see if they are in fact ST6. However, samples have been sent to the NICD but they are yet to be enumerated and unlikely to be completed. It is important that VPH-Gauteng completes the serotyping to satisfy ourselves that we do not have the ST6 in the Gauteng abattoirs. GVS should also introduce routine bacteriological sampling in the abattoir audits system, over and above any testing and culturing which the abattoir owners may be undertaking. State Veterinary Services should also seriously consider new approaches to safeguarding the animal protein food chain, such as pathogen reduction approaches and risk based methodologies in abattoir hygiene monitoring.

The food safety environment in South Africa Is characterised by multiple regulatory authorities whose mandates typically overlap in several instances. Efforts to restructure the food safety regulatory environment have been made in the past but none have really gained traction. Even within government, various aspects of food safety control fall under several departments whose collaborative efforts and information sharing efforts are not always consistent. The recent Listeria outbreak which has been the largest recorded Listeria outbreak has brought this issue to the fore once more as it became clear that only a concerted multi-sectorial, multi-departmental approach would yield positive long term returns. It is quite clear that the structure of food safety control in South Africa needs to be re-examined in order to be responsive and pro-active.

Cattle Production Management Practices Predisposing Animals to the Incidences of Reproductive Conditions in Small Scale Farming

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It is important to supply adequate resources required for pregnancy to reduce the risks of calving problems. The incidence of reproductive disorder during parturition is a complex subject matter influenced by factors associated with management and systemic health. Planning for an effective reproduction begins prior parturition during the dry period. This study aimed to identify management practices which predispose cows to reproductive conditions in communal farming.
The sample size was determined from statistical data obtained from the Molopo state veterinary offices of Mafikeng areas. The Rao Soft calculator was used to determine the sample size of 135 from the population of communal farmers. The sample groups consisted of farmers which encountered incidences of dystocia (n=22), downer cow syndrome (n=26), retained placenta (n=31), vaginal prolapse (n= 23) and abortion (n=33) in their herds. Data were collected during farm visits following cases reported to the Dale Beighle animal hospital in the North West University. Structured questionnaires were used to obtain demographic information from the farmers as well as information about the cows.

The data were analysed using SPSS version 23. Pie charts were used to outline the frequencies. The Chi-square tests indicated that the reproductive condition encountered is significantly associated (p-value < 0.05) to the cow breed, parity, feeding system, whether the farmer has heard about Brucellosis and the frequency of getting animals checked by a veterinarian. The cows from the farms whose farmers have heard about Brucellosis stand a relatively high (34%) chance of experiencing Downer syndrome and a relatively high chance of experiencing retained placenta. Similarly, the cows from farms whose farmers have never heard of Brucellosis stand a relatively high chance of experiencing Abortion (34.1%) and Dystocia (17%).

There is a vital necessity to embark on identifying significant indicators for the reproductive conditions on a broader scale in communal farming. It is equally important to ensure implementation by closely monitoring of disease surveillance and control programs through animal care practitioners through collaborations between the research institutions and the government as a means to improve breeding selection practices, nutritional balance and overall proper herd health management in rural farms.

Prevalence of Campylobacteriosis and Trichomoniasis in Communal Cattle of Umgungundlovu, Kwa-Zulu Natal

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The purpose of this study was to determine the prevalence of bovine genital campylobacteriosis and trichomoniasis, caused by *Campylobacter fetus*[1] and *Tritrichomonas foetus*[2] respectively. This study was conducted under direct instruction and supervision by staff of Allerton Provincial Veterinary Laboratory and Pietermaritzburg State Vet Office, Kwa-Zulu Natal, South Africa.

The cattle in uMgungundlovu Municipal District were selected as the study population. Allerton is located within this district, which eased handling and transport of samples. Multi-stage cluster sampling[5] was used to calculate a cluster size of 17 diptanks and a sample size of 79 bulls. Criteria for selecting bulls required that owner or livestock association member consent was obtained for the study, minimum age was three years, dominant breeding status and a history of abortions or other reproductive failures in that herd – if such information was available.

Campylobacter fetus and *Tritrichomonas foetus* were isolated from preputial scrapings in sterile phosphate-buffered saline transport medium using Skirrow's medium and *Trichomonas* broth in bijou respectively. Culture results were confirmed with polymerase chain reaction (PCR), including gel electrophoresis[4]. These tests were done according to Allerton's standard operational procedures. A total of 63 bulls were sampled from 16 diptanks of which four tested positive on PCR for *Tritrichomonas foetus.* All *Campylobacter fetus* samples tested negative. Therefore, a true prevalence of *T. foetus* in uMgungundlovu was found to be 6.22%.

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Risk Factors for Infection of Bovine Tuberculosis in Cattle and Communal Farmers Living at the Wildlife-Livestock-Human Interface In Kwa-Zulu Natal, South Africa

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Cattle are the reservoir of Mycobacterium bovis (*M. bovis*) but many other domestic and wild animals including humans can be affected by bovine tuberculosis (bTB). In South Africa, bTB in cattle is only partly controlled and the African buffalo (*Syncerus caffer*) is the reservoir of *M. bovis* in several ecosystems infected with *M. bovis* and gives rise to a complex wildlife-livestock-human interface which increases the risk of *M. bovis* transmission. A collaborative study with the Department of Health was conducted to investigate the occurrence of *M. bovis* in members of 75 households associated with bTB infected herds. The initial screening was done using the GeneXpert for the Mycobacterium tuberculosis complex (MTC) followed by speciation using molecular techniques. A questionnaire was administered to the farmers to investigate the communal farmers' risk practices to bTB transmission among cattle and to humans. Out of 71 respondents 63 (89%) of the individuals did not know about bovine tuberculosis in wildlife and 61(86%) of the respondents were aware of bTB in cattle but 70% of these were not aware of its transmission to human. There is regular contact of cattle from different herds during grazing as all the farmers use communal pastures. The frequent consumption of unpasteurized milk as fermented milk (amasi) was identified as am important risk factor for bTB transmission to humans. The study has demonstrated poor knowledge of most cattle owners concerning bTB and its transmission pathways among people, livestock and wildlife. This is part of a One Health study that was designed to assess the role of *M. bovis* at the interface of cattle, human and wildlife.

Isolation of *Brucella Melitensis* Biotypes 2 and 3 from Slaughter Cattle in South Africa

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Isolation of *Brucella melitensis* has been demonstrated and reported in many parts of the world (B Lopes et al., 2010). *Brucella melitensis* is primarily a pathogen found in goats and sheep, but occasionally, it can be found in cattle. In South Africa, this organism has been responsible for outbreaks of brucellosis in goats and has also been reported to be the cause of brucellosis in humans (Seifu et al., 2004).This study reports for the first time in South Africa, the detection and isolation of *Brucella melitensis* from tissue samples of slaughter cattle from abattoirs in the Gauteng province of the country. Two hundred serum and corresponding tissue samples (lymph nodes, spleen and liver) were collected from 200 slaughter cattle between September 2016 and April 2017. Serological tests using the Rose Bengal Test (RBT) and indirect enzyme linked immunosorbent assay (iELISA) were conducted on serum samples from these cattle. The genus-specific 16SrDNA to 23S rDNA interspacer region (ITS) PCR assay was used to detect *Brucella* DNA in the lymphatic, liver and splenic tissues of the ELISA positives(Keid et al., 2007). All ITS-positive tissues were inoculated on both standard Farrells and modified CITA media. Isolates morphologically identified as *Brucella* colonies were subjected to biochemical tests for identification and biotyping at the South African National Accreditation System (SANAS)-accredited laboratory of the Onderstepoort Veterinary Research, South Africa using approved protocol (Ribeiro and Herr, 1990). All isolates of *Brucella* spp. were again subjected to the same ITS PCR assay as described earlier and AMOS multiplex species PCR assay that detects the IS711 genes of the *Brucella abortus*, *Brucella melitensis*, *Brucella ovis*, *Brucella suis* (AMOS)(Bricker, 2002). Of the 200 sera tested, 22 (11%) and 11(5.5%) were positive for antibodies to *Brucella* spp. by RBT and iELISA respectively. ITS assay detected *Brucella* DNA in 9 (81.1%) of 11 tissues of iELISA-positive cattle while *Brucella* spp. were isolated from 7 (77.7%) of 9 ITS-positive samples. The AMOS PCR identified 4 (57%) of 7 isolates as *Brucella melitensis* and biotyping classified two isolates as *B. melitensis* biotype 3 and one isolate as *Brucella melitensis* biotype 2. The biotype of the fourth isolate could not be determined. It is well documented that *B. melitensis* is an important abortifacient agent of goats and sheep with associated economic losses (Emslie and Nel, 2002, McDermott and Arimi, 2002) and more importantly, a zoonotic pathogen, causing more severe clinical manifestation than all other *Brucella* spp. reported to infect humans. In South Africa, although *B. melitensis* strains have been isolated from sheep and goats (Caine et al., 2017), there is no documentation of infection by biotypes 2 and 3 of *B. melitensis* strains nor has there been a report of the pathogen infecting cattle in the country.The implication of these findings underscores the fact that humans can become infected with *Brucella melitensis* from infected cattle, either through drinking of unpasteurized milk or consumption of under cooked or uncooked meat products from these infected animals

(Adesiyun and Cazabon, 1996, Leclerc et al., 2002, Kuplulu and Sarimehmetoglu, 2004, Seifu et al., 2004, Garin-Bastuji and Verger, 1994). Another important public health implication is that the abattoir workers stand the risk of occupational-associated zoonotic infection from these *B. melitensis* infected slaughter cattle.

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Endemic Circulation of Rift Valley Fever Virus in Far Northern Kwazulu-Natal

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Rift Valley fever (RVF) is a mosquito-borne zoonotic disease characterized in South Africa by large epidemics amongst ruminant livestock at very long, irregular intervals, mainly in the central interior. However, the presence and patterns of occurrence of the virus in the eastern parts of the country are poorly known. This study aimed to determine whether RVF virus (RVFV) is circulating in cattle, goats or wildlife in far northern KwaZulu-Natal, close to the Mozambique border, and, if so, to estimate seroprevalence and incidence rate of seroconversion.Cross-sectional studies were performed in wildlife (nyala and impala; n=156) and in communally farmed cattle (n=423) and goats (n=104), followed by longitudinal follow-up of seronegative livestock (n=253) 9 times over 18 months, representing 116.2 animal-years at risk. Exposure to RVFV was assessed using the serum neutralization test. In seroconverting animals, multiple imputation was used to impute a random date of seroconversion between last negative and first positive tests, in order to provide an unbiased estimate of seroconversion rate over time. Incidence density was estimated and compared using Poisson models and seroconversion rate was plotted over time using the derivative of the kernel-smoothed Nelson-Aalen cumulative hazard estimator.

Initial overall seroprevalence was 34.0% (95%CI: 29.5-38.8) in cattle, 31.7% (95%CI: 22.9-41.6) in goats and 45.9% (95%CI: 37.9-54.0) in wildlife. Although it tended to increase with age, it was high in all age groups. Overall rate of seroconversion in cattle was 65.4 (95%CI: 50.3-85.1) and in goats 65.9 (95%CI: 42.5-102.3) per 100 animal-years, varying significantly between localities within a 10 km radius. Seroconversions were detected throughout the year, with incidence rate peaking during the high rainfall months of January to March.The high seroprevalence in all age groups and evidence of year-round viral circulation indicate a hyperendemic situation in the study area. This is the first study to directly estimate infection rate of RVFV in wildlife and livestock in an endemic area and provides the basis for further investigation of mechanisms for virus circulation in endemic areas and survival during interepidemic periods.

Key words: Rift Valley fever, vector-borne diseases, zoonoses, seroconversion, incidence density

Temporal Patterns of Anthrax Outbreaks and Cases Among Livestock in Lesotho over a Period of Eleven Years (2005-2016)

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Background: Although anthrax is endemic in Lesotho, limited information is available on the pattern of the disease among livestock. This study investigated temporal trends of anthrax outbreaks and cases among livestock in ten districts of Lesotho.
Material: Secondary data of anthrax outbreaks reported to the Department of Livestock Services between January 2005 and December 2016 was used for this study. Proportions of anthrax outbreaks and anthrax cases, and their corresponding 95% confidence interval were calculated and compared across year, season, month and region using Chi-square or Fisher's exact tests. A time-series analysis was conducted to evaluate the incidence of anthrax outbreaks and cases over an eleven-year period. All analyses were performed using SAS 9.4.

Results: A total of 38 outbreaks were reported over the study period, in the Lowlands districts of Lesotho. Maseru district reported significantly ($p<0.0001$) higher proportions of outbreaks (52.6%) and cases (70.2%) compared to other districts. Anthrax outbreaks (78.9%) and cases (95.1%) were significantly ($p=0.0004$) higher in the rainy season than the dry season. Five hundred and twenty-six ($n=526$) anthrax cases were reported, with cattle having significantly ($p<0.0001$) highest proportion of cases (70.3%). There were significantly ($p<0.0001$) more (35.9%) anthrax cases reported in 2008 compared to other years. February (30.8%) and April (30.2%) compared to other months had the highest proportions of anthrax cases reported ($p<0.0001$). There was no significant annual trend in disease outbreaks ($r=0.0282$; $p=0.6213$) and cases ($r=0.873$; $p=0.3512$).

Conclusion: The burden of anthrax in Lesotho is significantly higher in cattle. Anthrax outbreaks occur in the Lowlands districts and follow a seasonal pattern. Therefore, more effort should be targeted at curbing the disease in cattle and the Lowlands districts; Furthermore, there should be heightened monitoring of cases in the rainy season to ensure that resultant carcasses are disposed of appropriately to minimise future outbreaks.

Development of Improved Molecular and Serological Assays for Epidemiological Characterisation of Shuni Virus in Humans, Animals And Vectors

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Emerging and re-emerging infectious diseases are an important global problem as they can lead to major disease outbreaks. Arboviruses (arthropod-borne viral diseases) contribute significantly to such diseases worldwide. Shuni virus (SHUV) is a suspected re-emerging arbovirus of the *Orthobunyavirus* genus in the *Bunyaviridae family.* It has previously been implicated to cause neurological diseases in horses, livestock, wildlife and potentially humans.

To determine the epidemiology and disease burden of SHUV, SHUV specific molecular tests were performed and evaluated for diagnostic and screening purposes. This was achieved by comparing the specificity and sensitivity of three PCRs *i.e.* SHUV specific HybProbe nested PCR, a TaqMan Orthubunyavirus one-step genus specific PCR and a TaqMan one-step SHUV specific PCR.

The TaqMan Orthubunyavirus one-step genus specific PCR was as sensitive as the SHUV specific HybProbe nested PCR as it could detect up to the lowest dilution (log 6). Therefore, it was used as an additional screening method. A total of 19/1191 (1.7%) horses were identified as SHUV positive between 2009 to 2016 using the SHUV specific HybProbe nested PCR. Surveillance is still on-going using the TaqMan Orthubunyavirus one-step genus specific PCR; however, no new cases have been detected since 2016.

This demonstrates that SHUV is circulating, however, more research is needed on reservoir hosts, vectors and transmission cycle. Furthermore, its importance as a human pathogen still needs to be determined by further investigating the incidence of SHUV cases and its disease presentation.

Multinomial Logistic Regression in Infection: Application For Diagnosis of Canine Clinical Cases of Multi Species *Staphylococcus* Infetion

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Veterinary clinicians are faced with the challenge of making clinical diagnoses on daily bases. Drawing up a list of deferential diagnoses is the first step unless the disease has pathognomonic clinical signs. This list often consists of more than two possible outcomes in which the clinician can choose from. If bacterial infection is suspected, in addition to the clinical presentation, the results of culture may be needed to make a definitive diagnosis. However, the laboratory turnaround time for isolation, characterization of species and establishing resistance profile of the isolates is often long. Therefore, clinicians are forced to rely on clinical presentation and empirical treatment in order to manage the patient. Studies have shown that bacterial species with more than one serotype differ in pathogenicity and response to treatment. Therefore, a wrong diagnosis can negatively affect patient care and recovery. For example, pyoderma in dogs can be caused by either *S. pseudintermedius*, *S. aureus* or other species. However, *S. aureus* compared to other species is more virulent and has a higher degree of resistance to antimicrobial drugs commonly used for treatment of pyoderma. Multinomial logistic regression models have been used widely in public health to model polytomous outcome variables. The predictor variables in a multinomial logistic model may be nominal, ordinal, interval or ratio. Similar to binary logistic regression, the multinomial logistic regression uses maximum likelihood to estimate model parameters. We apply a multinomial logistic regression model to investigate the associations between a polytomous outcome (*S. aureus, S. pseudintermedius*, negative outcome) and a set of predictors (breed, season, year, sex, age and specimen type). The outputs of these kinds of models are useful for guiding clinical decisions in the treatment of *Staphylococcus* spp. infections that are known to exhibit differences in predictors, pathogenicity and antimicrobial drug resistance patterns.

Validation of an Indirect Immunoperoxidase Test for Rabies Virus in Domestic and Wildlife Species in South Africa

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Rabies virus is a member of the genus *Lyssavirus*, in the family *Rhabdoviridae*. This fatal but preventable disease is of significant public health and veterinary importance in the developing world. The only reliable method of detection is to determine the presence of the virus via immunodetection of viral antigen in the central nervous system (CNS) tissue following death of the animal. Rapid diagnosis is key in preventing human deaths due to rabies and to implement effective control measures in the field. The purpose of this study was to validate the rabies indirect chromogen-immunohistochemistry test (IC-IHC), and to estimate its diagnostic sensitivity and specificity on routine specimens submitted for the rabies direct fluorescent antibody test (FAT) collected in South Africa.

The IC-IHC test was performed on CNS tissues obtained from a variety of mammalian host species commonly submitted for rabies diagnostic testing in South Africa, samples from domestic dogs and cattle were submitted in the greatest number to the two accredited FAT rabies laboratories in South Africa during the study period from 2013 - 2017. Specimens from wild animal species that included, but were not limited to, jackal, wild dog, hyena, aardwolf, leopard, lion and mongoose were also tested. One hundred ninety-nine cases were evaluated, of which 99 were positive and 100 were negative for rabies on FAT. The IC-IHC test results were compared to those of the gold standard to determine its sensitivity (Se) and specificity (Sp). The overall Se and Sp for the rabies IC-IHC test was 98 % (95 % confidence interval (CI): 93 % - 100 %) and 99 % (95 % CI: 95 % - 100 %) respectively.

As part of the validation study, we investigated the effects of autolysis (30.7 % of test cases were severely autolysed and 43.7 % moderately autolysed), extended time in formalin (samples fixed in formalin for up to a year) and freezing on the diagnostic Se and Sp of the IC-IHC. It was determined that the brainstem and the thalamus were the best samples to collect for rabies virus detection using the IC-IHC test in all species.

The IC-IHC test used in the current study is an excellent diagnostic test for rabies in South Africa and compares well with the FAT. The test has the added benefit of working on formalin fixed tissues and since formalin inactivates the virus, the safety of field and laboratory staff and couriers is assured. Also, if the sample examined with the IC-IHC is negative, there is the added benefit of histopathologic examination of the brain tissue to look for other differential diagnoses.

A Bioeconomic Model for the Optimization of Local and Regional Canine Rabies Control

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The World Health Organization estimates that about 55,000 people die from rabies each year. Although the mortality risk from rabies infection is relatively small compared to some other diseases, rabies is distinctive because infection in humans is both easily prevented with vaccination and easily treated after exposure. Additionally, a low-cost, effective vaccine is available for managing and eliminating the disease in dogs, which are the primary source of human exposure in much of the developing world. Our focus in this paper is based on two observations. First, the elimination of human exposure in the developing world results from the elimination of the disease in dogs. Eliminating the disease in dogs provides ongoing benefits by avoiding the relatively high and unending costs of human treatment. Furthermore, in any population, there will always be people who are unwilling or unable to obtain PEP in response to a potential exposure. Thus, elimination of the disease in dogs will reduce human mortality even if access to PEP is widespread. Our second observation is that canine rabies management funding and planning is often haphazard. Coordinated international efforts are rare, and even efforts within a single country may not be well-coordinated. With these observations in mind, and given the near universal lack of sufficient management resources, our goal was to develop a tool that can be used to maximize the impact of whatever canine rabies management resources are available at the local and regional levels.

The tool we have developed is a bioeconomic model that can be accessed through a web-based graphical user interface (https://bioecon.shinyapps.io/CanineRabiesWebApp) as well as a complementary command-line interface. The model is an individual-based, stochastic simulation model that explicitly accounts for the links between management effort, management cost, and biological outcomes. Additionally, our objective was to construct a model that (1) accounts for population and disease dynamics, (2) allows vaccination, permanent sterilization, temporary contraception, and removal, (3) allows strategies to vary temporally and demographically, (4) allows combination strategies, and (5) is flexible enough to allow parameterization for many different canine rabies management scenarios. Although all our code is freely available (https://github.com/anderaa/BioEcon_CanineRabies) and can be modified by a user if desired, our model can be used in applied settings by users without computer programming experience.

Pathological Survey of Diseases of African Buffalo in South Africa

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African buffalo (*Syncerus caffer*) are one of the most iconic species of South African megafauna. There are approximately 2470 registered buffalo farms with 45,000 – 60,000 farmed buffalo and an additional 40,000 – 50,000 free-ranging buffalo within the Greater Kruger National Park Complex. Disease surveillance is invaluable in protecting both animal and public health and food security. A retrospective study of pathology cases in 429 buffalo (2001-2015) was performed to assess the spectrum of lesions seen in farmed (n=251), free-ranging (n=161) and zoo (n=19) buffalo in South Africa to provide a frame of reference for veterinarians, pathologists, wildlife biologists, and managers of game reserves.

This study confirmed that African buffalo are susceptible to many of the same diseases as domestic cattle in South Africa such as Rift Valley hepatitis, theileriosis, bacterial and viral enteritis, haemorrhagic septicaemia, coccidiosis, ruminal acidosis, salt toxicity, polioencephalomalacia, and exertional and nutritional myopathy. Increased disease surveillance and intensive management practices likely accounted for the increased prevalence of infectious, nutritional, senile and congenital disease in zoo and farmed animals, compared to free-ranging ones. Malignant catarrhal fever (3.5%) was significantly more prevalent in farmed buffalo than other groups, possibly due to increased contact with ruminant species to which they typically have little contact in a free-ranging situation. Protein/energy malnutrition (4.4%) was significantly more common in zoo animals. Tuberculosis (12.8%) and lymph nodes draining areas of haemorrhage (8.2%) were significantly more common in free-ranging buffalo since these buffalo were mainly shot for tuberculosis surveillance. Skeletal muscle Sarcocystis infestation (0.5%) was only diagnosed in this group since the lifecycle of this parasite is not completed in zoo and farmed animals.

These results likely do not indicate true prevalence of fatal disease since not all mortalities are submitted for pathological examination; and since cause of death could not be determined in 25.2% of cases due to autolysis, restricted tissue submission and lack of supporting clinical data. Continued and diligent surveillance of captive, farmed, and free-ranging populations alike will be valuable to inform the management of captive buffalo.

How Sure are you of that Result?

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Primary binding assays such as the indirect immunofluorescence assay or the enzyme-linked immunosorbent assay (ELISA) are serological methods that have been used to effectively screen for many diseases in humans and animals. These assays depend on reagents that can recognise antibodies of the target species. The indirect ELISA, for example, requires an enzyme conjugate, which is specific to the antibodies (IgG) of the target species, also referred to as an *anti-species immunoglobulin*. Conjugates are commercially available for all common domestic species and even for the predominant wildlife species in the northern hemisphere. The African wildlife species are, however, extremely diverse and anti-species immunoglobulins are not readily available, which renders primary binding assays that depend on these conjugates largely unavailable for these species.

Using, as an example, a study to evaluate the binding of commercially available horseradish peroxidase (HRP) conjugated recombinant protein A/G with IgG of 27 African herbivore wildlife species, the effect of sample size and intraspecies variation on the 95% confidence intervals are illustrated. For this study a simple direct ELISA was performed on serum samples of 10 animals from each wildlife species and the binding with recombinant protein A/G was expressed relative to the response to a homogenous bovine serum control. The effect of poor laboratory technique will be illustrated through an example where invalid controls impacted on the validity of results.

In addition to the evaluation of binding capacity, a technique described in human immunology to evaluate the strength of these molecular bonds was explored during this study and the relative avidity index (RAI), quantifying the effect of a chaotropic agent, was calculated. A usefulness index was proposed to rate different candidate conjugates tested for possible use in wildlife species. Thirteen wildlife species, namely elephant, Burchell's mountain zebra, black and white rhinoceros, warthog, hippopotamus, tsessebe, blesbok, springbok, steenbok, African buffalo, duiker, sable antelope and common reedbuck, performed better than or equal to the bovine control with the recombinant protein A/G: HRP, while the rest of the species bound significantly less than the bovine control. Subject to proper assay validation and the calculation of appropriate cut-off values to attain required test sensitivity and specificity in accordance with the purpose of testing, whereby test results can accurately be interpreted, recombinant protein A/G: HRP may be considered for use in immunosorbent assays developed for the listed wildlife species, where binding with species-specific IgG is required.

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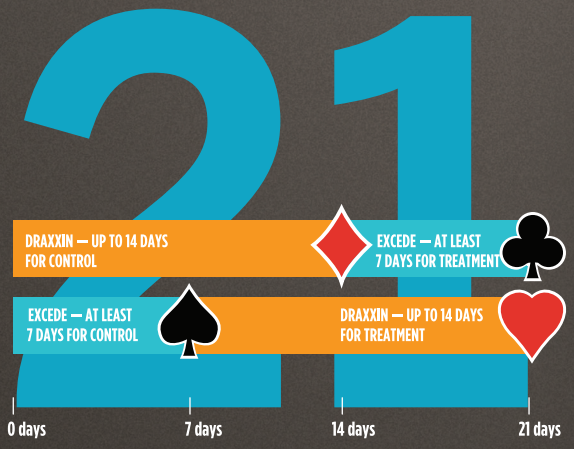

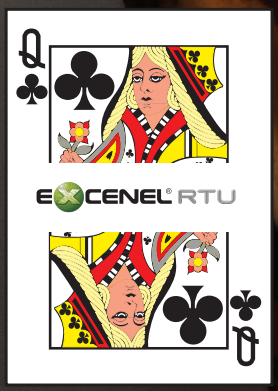

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