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### CONTINUING EDUCATION PRESENTERS

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Head: FAO Reference Centre for Veterinary Epidemiology  
Royal Veterinary College  
London

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# **CONTINUING EDUCATION PRESENTATION: SPATIAL ANALYSIS IN VETERINARY EPIDEMIOLOGY**

Pfeiffer, D<sup>1</sup>

## **SUMMARY**

No summary available at time of publication.

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# BOVINE BRUCELLOSIS IN SOUTH AFRICA

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## ABSTRACT

Confirmation of Bovine brucellosis by culture in South Africa was reported by GN Hall in 1913 and again in 1918 by EM Robinson. The first report of undulant fever in man was reported by LEW Bevan in 1922 following an outbreak of infectious abortion in cattle, and again in 1932 by PD Strachan. In 1970 B. abortus Strain 19 was introduced into the country for vaccination of calves between the ages of 4 – 8 months. On 12 November 1976 the Bovine brucellosis accreditation scheme was implemented and this was followed on 1 July 1977 by the Diagnostic Scheme. On 9 December 1988 the Bovine Brucellosis Scheme appeared in Regulation 2483. In 2002 The MSD RB51 vaccine became commercially available in South Africa.

With legislation and availability of vaccines to increase herd immunity, bovine brucellosis has not been eradicated from the country and it appears that the situation is worsening. Since 2003 more than 250 outbreaks have been reported a year (range from 263 – 416) with 78 outbreaks reported in the first two months of 2014. Where are the authorities, veterinary profession and farming community going wrong? Knowledge of the disease, control policies, vaccination policy, movement control, risk analysis, diagnosis and other factors need to be explored to contain, control and finally eradicate the disease from the country.

A national census of livestock and surveillance should be introduced to determine the true prevalence of the disease in the country. With this information the prevalence can be monitored and evaluated to determine the success of control and eradication activities implemented. Census will enable authorities to plan for vaccination campaigns to improve the immunity of the national herd against bovine brucellosis. High risk areas will be identified where intensive test and cull operations with increased vaccination could be introduced. A national awareness campaign to improve the population's knowledge of the disease should be implemented. Farmer's awareness of biosecurity will help production at all levels. All these activities cannot be the responsibility of veterinary services alone but will have to be embraced by farmers, industry and related fields to ensure the successful control and eventual eradication of the disease from the country. The situation amongst the human population is not known but is thought to be grossly under diagnosed.

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# DYNAMICS OF AN OWNED, FREE-ROAMING DOG POPULATION: IMPLICATIONS FOR RABIES CONTROL

Knobel, D.L.<sup>1\*</sup>, Akerele, O.A. & Conan, A.

## ABSTRACT

Rabies in free-roaming domestic dog populations is a serious public health threat in underserved communities in South Africa and elsewhere on the continent. Rabies in dog populations (and consequently in humans) can be controlled and in certain circumstances eliminated through the mass vaccination of dogs against the virus. The control of rabies through vaccination relies on maintaining population-level vaccination coverage above a critical threshold. This goal is hampered by the rapid turnover of dogs in free-roaming populations in underserved areas. The problem is compounded by the fact that mass dog rabies vaccination in these areas is usually implemented in annual (or less frequent) campaigns, between which the vaccination coverage in the population declines as vaccinated dogs die and unvaccinated dogs enter the population through birth or in-migration.

Understanding the population dynamics of free-roaming dog populations, particularly the core demographic rates of birth, death and migration, is therefore essential for the effective implementation of mass vaccination to control rabies in underserved communities. Despite the ubiquity of free-roaming dogs in sub-Saharan Africa, little is known about the demographic rates of these populations, or the factors that affect them. Evidence from a number of studies in the region has shown that, despite appearances, the vast majority of dogs (>90%) in these populations are owned. Demographic surveillance of dog populations is therefore possible through ongoing monitoring of individuals within households. Here, we report the results of a demographic surveillance system in an owned, largely free-roaming dog population in a community of around 10,000 people in 2,000 households in Hluvukani, Bushbuckridge Local Municipality, Mpumalanga Province. Following an initial census, regular visits (every 3-4 months on average) were made to all households within the designated surveillance area. During these visits, information on individual dogs was updated, including key demographic events. Data spanning 24 months (1st January 2012 through 1st January 2014) is presented.

During the 24-month period, the total population of owned dogs declined by 10%, from 792 dogs to 710 dogs. However, there was a substantial fluctuation in this population, reaching a peak of 955 dogs in the last quarter of 2012 before declining sharply. The annual growth rate was 19% in 2012 and -24% in 2013. Birth rates were extremely high: the crude birth rate was 451 dogs per 1,000 dog-years in 2012 and 314 dogs per 1,000 dog-years in 2013. There is evidence of seasonality in birth rates, reaching an annual peak in autumn/winter. The crude death rate was 408 dogs per 1,000 dog-years in 2012 and 569 dogs per 1,000 dog-years in 2013. There was a sharp spike in the mortality rate in the second quarter of 2013. The net rate of in-migration of dogs into the study area increased in 2013, reaching a peak in the third quarter of that year. The sex ratio of the population was strongly male-biased. It remained stable during 2012, ranging from 1.37 to 1.39 male dogs per female, but increased steadily in 2013 to 1.75 by the end of the year.

Our findings show that this is a highly dynamic dog population, with rapid turnover and significant heterogeneity in demographic rates over time and across segments of the population. We demonstrate that, even in the face of this high turnover, routinely achieving 70% vaccination coverage against rabies during annual mass dog vaccination campaigns is sufficient to maintain vaccination coverage above the critical threshold, interrupting transmission of the disease and ultimately leading to its elimination from the population.

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# RISK OF HUMAN INFECTION WITH AVIAN INFLUENZA H7N1 AND H5N2 STRAINS DURING AN OUTBREAK IN OSTRICHES IN SOUTH AFRICA, 2012

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## ABSTRACT

Several avian influenza (AI) outbreaks have been reported in ostriches in the Western Cape Province of South Africa during the last 10 years, including highly pathogenic AI H5N2 in 2011, and low pathogenic AI H7N1 in 2012. Neither of these outbreaks led to an increase in ostrich deaths. Movement control and large-scale culling followed the 2011 H5N2 outbreak while movement control only was initiated during the 2012 H7N1 outbreak. We conducted a sero-survey among individuals involved in control efforts in 2012 to identify risk factors for human infection.

Sera and demographic data were collected in August 2012 under informed consent from veterinarians, laboratory workers, farm workers, and ostrich abattoir workers (n=66) involved in the 2012 H7N1 outbreak and state-veterinarians from across the country. Antibodies to H5 and H7 were measured by hemagglutination inhibition assays (HAI) and titres >1:20 confirmed by micro-neutralisation assays (MNA). MNA of >1:40 was considered positive.

We confirmed MNA antibody titres > 1:40 to H5 in one (1.5%) of 66 study subjects, a researcher who handled H5 infected ostriches in the Western Cape in 2011. Eight of the 66 subjects (12.6%) were positive for H7 of whom, 14.6% (n=6/41) were abattoir workers (95% CI (5.5-29.2)); 1/10(10%) veterinarians (95% CI (0.3-52.6)) and 1/4 (95% CI (0.6-80.6) laboratory workers (p=0.752). The veterinarian who tested positive for H7 reported conjunctivitis. In the group of state veterinarians from across the country, 11.1% (n=4/36) were positive for H7 and none for H5. None were from the Western Cape where ostrich outbreaks occurred however 1/4 investigated wild bird fatalities where H7 were confirmed, 1/4 inspected a quarantine station where H7 AI was identified in export birds and 1/4 inspected poultry farms in KwaZulu Natal provinces where AI outbreaks occurred which may have been the source of infections.

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## BRUCELLOSIS IN CATTLE, GAUTENG, 2007-2013

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### ABSTRACT

Brucellosis is a worldwide, zoonotic disease of economic importance. The prevalence of bovine brucellosis has increased in recent years in South Africa despite a national control programme utilising serological surveillance, test-and-slaughter and preventative vaccination. There is a paucity of published data on risk factors for both cattle and human health, and programme optimisation. This study is the first in a series to evaluate and mitigate the economic and zoonotic impact of brucellosis in Gauteng province, the smallest but most populous province in the country.

We analysed data from all milk and blood samples ( $n = 422\ 018$ ; 7376 batches (representing “herds”)) tested by the reference laboratory (Onderstepoort Veterinary Institute) between 2007 and 2013. Data was analysed in EXCEL (Microsoft 2010) and RStudio (version 0.98.597). An average of 60 289 samples were tested per year of which 1.2% were milk samples. Herd prevalence using blood samples only, was found to be 36.6% (min: 32.8%; max: 40.1%). 10.8% of dairy herds tested in 2013 were positive. The prevalence in cattle fluctuated between a low of 1.92% (2012) and 2.59% (2010) with an average of 2.33% for the period. 80% of CFTs had titres greater than 60IU/ml. Increasing herd size was a significant risk factor (Chi-square for trend = 1210.7,  $p < 0.05$ ; OR for herd size 20 to 100 = 3.36 (95% CI: 3.0 - 3.7); OR for herd size > 100 = 9.01 (95% CI: 7.8 - 10.4) that of smaller herds) despite herd sizes, 0 to 20, and 20 to 100 representing 56.0% and 30.5% of the sample size respectively.

This study emphasised the need to determine the zoonotic impact of brucellosis in Gauteng and to evaluate the current control and surveillance strategy. It also motivates an examination into risk factors associated with herd-level prevalence. We discuss the implications of this study and the further analyses that are underway to evaluate the representativeness of the herds tested.

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# **SPILOVER, SPILLBACK AND TRANSLOCATION OF BOVINE TUBERCULOSIS IN SOUTH AFRICA**

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## **ABSTRACT**

Bovine tuberculosis caused by *Mycobacterium bovis* (*M. bovis*) is an economically important disease of livestock and wildlife. Currently *M. bovis* has been diagnosed in 21 different wildlife species in South Africa, including the Greater Kruger National Park Complex (GKNPC), Hluhluwe-iMfolozi Park and several private game farms and conservation areas. The epidemiology of BTB in livestock and wildlife is intertwined as *M. bovis* is a multi-host pathogen which thrives at the wildlife/livestock/ human interface.

One of the major concerns of BTB infection in the KNP has been the spillback of BTB from wildlife to surrounding livestock populations. Following nearly 20 years of intermittent testing of cattle along the western and southern boundaries without evidence of *M. bovis* presence, a recent cross-sectional prevalence survey in the Mnisi area of the Bushbuckridge district revealed a BTB prevalence of 0.34% in this cattle population. Only one causative *M. bovis* strain was isolated from all four slaughtered animals which matched the KNP endemic *M. bovis* strain.

Apart from transmission at this geographical wildlife/livestock interface there is new evidence that large scale translocation of plains game which is not subject to pre-movement testing for BTB testing, has led to the creation of a man-made wildlife/wildlife interface, effectively facilitating *M. bovis* transmission over large distances in the country.

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## EMERGENCE OF GENETIC VARIANTS OF SAT2 FOOT-AND-MOUTH DISEASE VIRUSES AT THE WILDLIFE/LIVESTOCK INTERFACE IN SOUTH AFRICA

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### ABSTRACT

Foot-and-mouth disease (FMD), of which FMD virus (FMDV) is the causative agent, is a highly contagious, acute infection of cloven-hoofed animals. The disease is a compulsory notifiable disease with global distribution. Although FMD is characterised by low mortality rates, the disease has a major economic impact on the livestock industry. Rapid and accurate diagnosis of FMD is a prerequisite for effective control of the disease and is based on a combination of clinical, epidemiological and laboratory observations. In sub-Saharan Africa, control of the disease is complicated by the extensive variability of the South African Territories (SAT) type viruses, which exist as distinct genetic and antigenic variants in different geographical regions. The SAT types are endemic to the most north-eastern corner of South Africa including the Kruger National Park (KNP), where it is maintained through persistent infections of African buffalo. The occurrence of FMDV within the KNP constitutes a continual threat to the livestock industry in South Africa.

Since 2000, several outbreaks of FMD have occurred in southern Africa emphasising the need for ongoing disease surveillance. An outbreak of FMD occurred in 2013/2014 and SAT2 type viruses were isolated from cattle adjacent to the KNP. Following initial screening using a fast one-step PCR, the viral RNA was amplified by RT-PCR, purified and used for sequencing. The nucleotide and deduced amino acid data was analysed for genetic and phylogenetic comparisons. Phylogenetic analysis of the recent outbreak viruses revealed their genetic relatedness to other SAT2 isolates from topotype I (South Africa, Zimbabwe and Mozambique). The recent SAT2 outbreak viruses are genetically distinct from previously isolated viruses (2011 and 2012) and form an outgroup within the topotype I viruses. These results confirm the genetic variability of SAT2 viruses emphasising that continuous investigation of the genetic characterisation of field viruses is important with regards to determining the occurrence of new virus strains, epidemiological surveillance aspects and vaccination.

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# A VALUE-CHAIN APPROACH TO REDUCE FOOT-AND-MOUTH DISEASE TRANSMISSION RISK ASSOCIATED WITH DEBONED BEEF PRODUCED FROM AN ENDEMIC REGION

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## ABSTRACT

Foot-and-mouth disease (FMD) is an economically important disease of livestock and a global threat to trade in livestock and livestock products. The Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement") entered into force with the establishment of the World Trade Organization (WTO) on 1 January 1995. The SPS Agreement encourages the wider use of systematic risk assessment among all WTO member governments and stipulates that if another country can show that the measures it applies provide the same level of health protection, these should be accepted as equivalent. Article 8.6.26 of the Terrestrial Animal Health Code produced by The World Organisation for Animal Health (OIE) contains the international recommendations for the importation of fresh beef from FMD infected countries or zones. The objective of this study was to determine if a value-chain approach could reduce the risk of FMD virus transmission via fresh deboned beef to an equivalent level as current OIE standards even when produced from an endemic region.

A value-chain approach for the reduction of FMD risk was developed for the Zambezi Region of Namibia. The approach combined commodity-based trade and hazard analysis and critical control points (HACCP) principles to integrate the management of all sanitary risks, i.e. those associated with food safety and transboundary animal diseases, FMD in particular. To estimate risk reduction in respect of FMD, a quantitative risk assessment (RA) was conducted that included both release- and exposure assessments with respect to FMD. Release and exposure risks were estimated independently for 19 cuts of beef; the quantity of FMD virus associated with each cut was estimated based on possible residual virus in muscle and the probability of lymphoid tissue contamination. Exposure assessment was based on feeding contaminated beef to pigs. The risk assessment was performed using a stochastic spreadsheet model based on published literature and expert opinion.

The system in current use in the Zambezi Region could potentially release FMD virus contaminated product once every two years compared to once every 5 years if the unmodified OIE Article 8.6.25 system were applied. The modified value chain system, on the other hand, would release contaminated product only once every 50 years. Infection of swine herds in trading partner countries purchasing fillets produced by the value chain management system would be expected to occur only once in 670 million years. This system has potential for benefiting many thousands of resource poor cattle farmers located in areas of Southern Africa where establishment of FMD-free zones is impossible.

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# **CONTINUING EDUCATION PRESENTATION: EMERGENCE OF ANTIMICROBIAL RESISTANCE IN ANIMALS**

Pfeiffer, D<sup>1</sup>

## **SUMMARY**

No summary available at time of publication

1. Head: Veterinary Epidemiology, Economics and Public Health Group  
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# **CONTINUING EDUCATION PRESENTATION: SYSTEMS PERSPECTIVE ON AVIAN INFLUENZA**

Pfeiffer, D<sup>1</sup>

## **SUMMARY**

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**SENSITIVITY AND SPECIFICITY OF REAL-TIME  
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REACTION, HISTOPATHOLOGY, AND  
IMMUNOHISTOCHEMICAL LABELING FOR THE DETECTION OF  
RIFT VALLEY FEVER VIRUS IN NATURALLY INFECTED CATTLE  
AND SHEEP**

Odendaal, L.<sup>1</sup>, Fosgate, G.T., Romito, M., Coetzer, J.A.W. & Clift, S.J.

**SYNOPSIS OF PUBLISHED ARTICLE**

Rift Valley fever (RVF) is a mosquito-borne disease caused by a virus of the family Bunyaviridae, genus Phlebovirus. In January 2010 suspicion of a pending RVF outbreak in South Africa was aroused following exceptionally heavy rains, abortion storms, extremely high mortality rates among young ruminants and gross and microscopic lesions typical for RVF in necropsied lambs. The diagnosis was confirmed using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC). Other diagnostic techniques were not used since they were either too lengthy to perform or too expensive. The World Animal Health Organization acknowledges histopathology, IHC and PCR as important methods to confirm a diagnosis of RVF but very limited information is available about the diagnostic accuracy of these tests.

In the absence of a perfect reference test, sensitivity (Se) and specificity (Sp) of qRT-PCR, histopathology, and IHC was estimated in a latent-class model using a Bayesian framework. Samples originated from the carcasses of naturally infected animals which were necropsied during the 2010 RVF outbreak. The most consistent and significant microscopic pathology for RVF occur in the liver. Therefore the liver was the organ most commonly submitted for testing and the only organ examined in this study. Three hundred and eighty cases from cattle (119) and sheep (261) were recovered for inclusion in the study. The Se and Sp of histopathology were estimated as 94.6% (95% CI: 91% - 97.2%) and 92.3% (95% CI: 87.6% - 95.8%) respectively. This was higher than expected proving the value of routine post mortem examinations and histopathology of liver samples. Notably experience from recent outbreaks of RVF in other countries revealed that the disease was not diagnosed in livestock until after human cases appeared. The Se and Sp of qRT-PCR were estimated as 97.4% (95% CI: 95.2% - 98.8%) and 71.7% (95% CI: 65% - 77.9%) respectively.

The extraordinary analytical sensitivity of PCR makes this test very susceptible to false positive reactions thus leading to an apparent reduced specificity during large-scale epidemics due to viral contamination at sampling sites and equipment in the field, necropsy facilities and testing laboratories. In addition, the format of sampling and submission is often inappropriate, with excessive amounts of material being submitted for testing, often in inadequate containers not suitably leak-proof. The processing of tissue samples for nucleic acid extraction is also more prone to contamination risks. The Se and Sp of IHC was estimated as 97.6% (95% CI: 93.9% - 99.8%) and 99.4% (95% CI: 96.9% - 100%) respectively. Considering the high sensitivity of the qRT-PCR and IHC both tests would be appropriate screening tests. Conversely, the high estimated Sp of IHC and the apparent reduced Sp of qRT-PCR suggests that IHC is an effective confirmatory test for RVF. IHC also has the added advantage that it requires formalin-fixed specimens which reduce the health risk to humans involved in the transport and testing of specimens. In addition, histopathology used in conjunction with IHC is the most feasible option to use in resource poor countries where biosecure reference laboratories are not available.

## PRELIMINARY RESULTS FROM SURVEY OF RAW MILK FROM INFORMAL MILK VENDORS IN PERI-URBAN HARARE, ZIMBABWE

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### ABSTRACT

In an ongoing study of the zoonotic risks associated with the raw milk sold by informal vendors in urban and peri-urban Harare, four potentially zoonotic bacterial species were identified during a roadside survey from January to March 2014. Thirty structured questionnaires were administered by interview to raw milk vendors at eight selling points around Harare. Raw milk was purchased from each vendor and submitted for bacteriological identification. These were positive for *Listeria monocytogenes* (10%), *Escherichia coli* (17%), *Staphylococcus aureus* (20%), *Streptococcus faecalis* (7%), and coagulase-negative *Staphylococcus* (7%). *Brucella spp.* were not identified in these milk samples despite over 53% of the vendors obtained their milk directly from the smallholder dairy farms, where no quality checks on the milk before sale would be expected. Associations between variables such as handling, storage and selling points of raw milk and milk TBC levels were not significant, except for the presence of *E.coli* in raw milk and the source of drinking water ( $\chi^2 = 8.75$ ,  $p=0.03$ ) with 77% of milk vendors using open well water. Many vendors sold milk that had high somatic cell count (19% CMT positive) and which was above the Zimbabwean legal limit for Total Bacterial Count of 500 000 cfu/ml (17%) and Total Coliform Count (13%).

This pilot survey noted the importance of this informal, unregulated market for the sale of milk from smallholder farms and for the supply of low priced product, but also identified issues of public health concern.

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# PREVALENCE OF ZONOTIC PATHOGENS IN BEEF PRODUCED IN GAUTENG ABATTOIRS

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## ABSTRACT

A study was conducted to determine the level of contamination and prevalence of zoonotic pathogens in beef produced at abattoirs in Gauteng Province, South Africa. Surface swabs were collected on different carcass parts and at various critical control points in randomly selected high (n = 2) and low (n = 2) throughput (HTP and LTP) beef abattoirs and subjected to microbiological analysis for total bacterial (TBC) counts of isolated organisms. *Salmonella enterica* serovar Dublin (1.05 %), *S. enterica* serovar Typhimurium (0.01 %), *S. enterica* serovar Muenster (4.43%), Shiga toxin producing *E. coli* (STEC) (2.08 %) and Vero toxin producing *E. coli* (VTEC) (5.42 %) were isolated in one LTP and the two HTP abattoirs. Beef samples from one LTP abattoir were negative to all the organisms that were isolated. The HTP (1.95) abattoirs had higher ( $P < 0.05$ ) log<sub>10</sub> (TBC) for Enterobacteriaceae than LTP (1.62). There was a progressive increase in log<sub>10</sub> (TBC) from the flaying point to the chillers for each sampled part across abattoir type. The brisket (2.80) had the highest ( $P < 0.05$ ) log<sub>10</sub> (TBC) followed by the flank (2.04) and neck (1.80). Log<sub>10</sub> (TBC) of all the sampled parts except the neck were higher ( $P < 0.05$ ) in the HTP than in the LTP abattoirs while neck samples in the HTP and LTP abattoirs had similar ( $P > 0.05$ ) log<sub>10</sub> (TBC). The prevalence of zoonotic organisms and level of contamination in Gauteng beef abattoirs was low. Though the prevalence of zoonotic pathogens was low, their presence on beef carcasses in the present study, highlight the need for institution of mitigation strategies to prevent food contamination.

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# MICROBIOLOGICAL QUALITY OF RAW POULTRY MEAT SLAUGHTERED IN ABATTOIRS OF GAUTENG PROVINCE, SOUTH AFRICA

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## ABSTRACT

The discipline of food safety is crucial for numerous groups including consumers, the food industry, and governments; hence pathogen detection and control are important in food microbiology. A plethora of programmes and interventions such as Hazard Analysis and Critical Control Points (HACCP), ISO 22000 and Good Production/ Manufacturing Practices are usually put in place to improve the safety of food. In order to ensure that these intervention programmes are effective, there is need to conduct monitoring, validation and surveillance studies to determine the presence of foodborne pathogens and indicators of fecal contamination.

The aim of this study was to evaluate the hygienic processing of poultry meat and detect foodborne pathogens at two high throughput and two low throughput poultry abattoirs in Gauteng province of South Africa. For this purpose, 114 chicken neck skin samples were randomly collected from poultry slaughter facilities between August 2012 and July 2013. The chicken neck skins were collected in sterile plastic bags, placed on ice and sent to the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI) Feed and Food Analysis laboratory for testing. The samples were analyzed for the prevalence of salmonella using an accredited method based on the South African Bureau of Standards, followed by serotyping. Total bacterial counts were enumerated using the pour plate technique.

Total bacterial counts were <100 000 cfu/cm<sup>2</sup> for all the samples tested. Using a one-way Analysis of Variance (ANOVA), with different types within the abattoirs, the Log means for total bacterial counts were significantly different among the four abattoirs ( $P < 0.05$ ). The means were separated using Fishers' unprotectd t, LSD test (Least significant difference). Likewise, Log means for total bacterial counts for high throughput and low throughput abattoirs were significantly different ( $P < 0.05$ ), with high throughput abattoirs showing higher levels of bacterial counts compared to low throughput abattoirs. Eight neck skins from the two high throughput abattoirs were positive for *Salmonella spp* (7.02%). The predominant serovars were *S. enteritidis* and *S. agona*, and these were present in equal proportions ( $n = 3$ ; 2.63% for each serovar). *Salmonella anatum* and *S. agona* were also present in equal proportions ( $n = 1$ ; 0.88% for each serovar). No neck skins tested positive for salmonella in low throughput abattoirs.

The results indicate that although the total viable counts were below the maximum limit of 100 000/ g as established by the South African 'Regulations Governing Microbiological Standards for Foodstuffs and Related Matters', there is need to improve hygiene during chicken slaughter in order to curb salmonella and to further reduce total bacterial counts as some of the bacterial counts were close to the cut-off value. In addition, the presence of *S. enteritidis*, which has zoonotic potential, highlights the need to tighten hygiene during chicken slaughter, particularly in high throughput abattoirs. The results also highlight the need for improved monitoring and control of salmonella at the poultry farms. This study necessitates extensive research among diverse poultry abattoirs and farms throughout South Africa and implementation of an effective "farm to fork" control programme.

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# COMPARATIVE GENOMICS OF *MANNHEIMIA HAEMOLYTICA* ISOLATES FROM RUMINANT LIVESTOCK IN SOUTH AFRICA

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## ABSTRACT

*Mannheimia haemolytica* is a Gram-negative weakly haemolytic coccobacillus and is the principal bacterial pathogen of respiratory disease in domestic and wild ruminants. Despite the huge economic significance of manheimiosis, there is lack of sufficient scientific information with regard to genetic markers that could clearly elucidate the molecular bases of serotype variability and allow understanding the pathogenesis and epidemiology of the different serotypes of *M. haemolytica* at genome level. Here we compare the nucleotide sequence data of *M. haemolytica* isolates in an effort to elucidate the genotypic polymorphism of South African isolates.

Twelve different isolates of *M. haemolytica* were retrieved from the archive for whole genome sequencing using the high-throughput GS FLX Illumina MiSeq® platform. Genomic DNA extractions were undertaken from the isolates using the Qiagen® DNA extraction kit and the purified high intact DNA was sequenced on an Illumina HiSeq® system (ARC- Biotechnology platform). The generated sequences were de novo assembled, the reads compared to each other, and then overlapped to build longer contiguous sequences. From the contigs, 16S RNA gene sequences of these isolates were extracted from the genome assemblies and compared with the sequence data from similar pathogens deposited in gene bank as USDA-ARS-USMARC-183 (Accession no. CP004752) and USDA-ARS-USMARC-185 (Accession no. CP004753). Phylogenetic analyses split the isolates into two distinct clades and further provided a tentative classification of these isolates. All the strains that clustered with USDA-ARS-USMARC-183 (CP004752) and USDA-ARS-USMARC-185 (CP004753) references were aligned to the respective references for genome-wide SNP genotype profiles.

This exercise revealed that there is on average 66% nucleotide sequence homology between the references and the individual strains and as a consequence, generating a very large number of SNPs with densities as high as 10 SNPs per kilo-base after quality filtering.

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# VIRULENCE PROFILES AND ANTIMICROBIAL RESISTANCE PATTERNS OF ESCHERICHIA COLI AMONG DIVERSE ANIMAL SPECIES IN SOUTH AFRICA

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## ABSTRACT

*Escherichia coli* is an important organism for both animals and humans because of its ability to cause diseases of the gastrointestinal tract, urinary tract infections and illnesses that are associated with the central nervous system. Virulence factors contribute to the ability of these bacteria to cause various diseases. Antimicrobial drugs have played an important role in treating and decreasing illness and death that is associated with infectious organisms such as *E. coli*. However, the vast use of antimicrobial treatment has led to multiple drug resistance; which complicates management of the illness and in the case of food production animals, the residual contamination of meat by antibiotics poses possible risk to consumers.

This study was aimed at determining the virulence profiles and antibiotic resistance of *E. coli* isolated from clinical and non-clinical samples among different animal species in South Africa. For this purpose, 130 *E. coli* isolates from different animal species including bovine (n = 56); equine (n = 14); porcine (n = 5); ovine (n = 5); caprine (n = 4); avian (n = 4); feline (n = 1); lion (n = 1); bok (n = 2); blue-wild bees (n = 2); and unidentified animal species (n = 37) were analysed using standard classical microbiological and molecular techniques. Three multiplex PCRs were used for detection of virulence genes encoding heat-labile (*LT*), heat-stable toxin a (*StA*), heat-stable toxin b (*STb*), shiga toxins (*Stx1*, *Stx2*, *Stx2e*), enteroaggregative heat-stable enterotoxin (*EAST-1*), adhesion involved in diffuse adherence 1 (*AIDA-1*) and porcine attaching and effacing-associated factor (*PAA*). Of the 130 *E. coli* isolates, 56 (43.1%) were positive for the tested virulence genes. Based on the positive virulence genes, the predominant group was EAEC which constituted 35.4% (n = 46), followed by STEC (6.2%; n = 8), and ETEC were the least predominant (1.5%; n = 2). A total of 70 virulence genes were observed and they were present in the following proportions: EAEC [*EAST-1* (n = 29; 41.4%), *PAA* (n = 27; 38.6%), *AIDA-1* (n = 3; .2%)], ETEC [*LT* (n = 2; 2.9%), *STb* (n = 2; 2.9%)], STEC [*Stx1* (n = 1; 1.4%), *Stx2* (n = 3; 4.3%), *Stx2e* (n = 3; 4.3%)], and *StA* was not detected in any of the isolates. The 56 *E. coli* isolates that harboured virulence genes were further characterized with respect to antibiotic resistance towards the following antibiotics: Ampicillin, Cefotaxime, Enrofloxacin, Florphenicol, Kanamycin, Lincomycin, Neomycin, Oxytetracycline, Penicillin, Polymyxin B and Trimethoprim. For this purpose, the modified Kirby-Bauer disk diffusion method was used. The zones of inhibition were measured and interpreted according to Clinical Laboratory Standards guidelines. All the isolates were resistant to Lincomycin. In addition, many of the *E. coli* isolates were resistant to Penicillin (n = 55; 98.2%), followed by Ampicillin (N=46; 82.1%), and Oxytetracycline (n = 22; 39.3%). Few of the 56 *E. coli* isolates were resistant to Neomycin (n = 3; 5.4%), Florphenicol and Polymyxin B (n = 2; 3.6%), Cefotaxime, Kanamycin and Trimethoprim (n = 1; 1.8%). All isolates were sensitive to Enrofloxacin. Multi-drug resistance (MDR) was observed in 55 (98.2%) isolates. The isolates that had highest number of virulence genes belonged to EAEC, and these *E. coli* also showed the highest occurrence of MDR. The presence of MDR *E. coli* could be a challenge for treatment of *E. coli* and may pose a risk to consumers.

The findings of this study highlight antibiotics that are useful in treatment of *E. coli*. In addition, the presence of a plethora of virulence genes among *E. coli* isolated from diverse animal species and high MDR in EAEC warrants extensive further investigation in future.

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# SEROPREVALENCE OF *BRUCELLA ABORTUS* IN CATTLE AT COMMUNAL DIPTANKS IN THE MNISI AREA, MPUMALANGA, SOUTH AFRICA

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## ABSTRACT

This study determined the seroprevalence of *Brucella abortus* in cattle at communal diptanks in the Mnisi area of Mpumalanga, South Africa. A total of 1 482 serum samples were collected from cattle at 19 diptanks and tested for antibodies against *B. abortus* using the Rose Bengal test (RBT). A total of 21 cattle tested positive for *B. abortus* antibody on RBT. Thirteen of the 21 samples that tested positive for *B. abortus* on RBT also tested positive on complement fixation test (CFT) and 12 of these tested positive on indirect enzyme linked immunosorbent assay (iELISA). The overall CFT confirmed seroprevalence of brucellosis in Mnisi area was 0.88%. The seroprevalence varied from 0% to 3.49% between diptanks. Seven diptanks recorded 0% prevalence. Antibody to *B. abortus* was found to be most prevalent in cattle from diptanks near the central part of the Mnisi area.

# THE KWAZULU NATAL PROVINCIAL BOVINE BRUCELLOSIS SERO-PREVALENCE SURVEY IN COMMUNAL CATTLE

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## BACKGROUND

Bovine brucellosis (BB) is a contagious disease affecting cattle worldwide and many other species of animals as well as humans. It is one of the biggest limitations to cattle farming in sub-Saharan Africa (SSA). The seroprevalence of BB in SSA subsistence farming communities is almost always above 5%. BB is considered endemic in most countries in SSA.

Previous studies in South Africa have estimated the incidence of BB at 6% and 3.7% in 1978 and 1980/1981 respectively. A survey conducted at a Cato Ridge abattoir in KwaZulu Natal (KZN) estimated BB at 1.3%. Based on these three studies evidence seems to suggest that BB is on the decline. However caution is required because all these studies were not completely based on epidemiological principles in the nature of their design. There is a strong suspicion that the seroprevalence of BB is very high amongst communal cattle in KZN.

## MATERIALS AND METHODS

### STUDY DESIGN

This was a cross sectional study conducted between June 2013 and December 2013.

### SAMPLE SIZE AND SAMPLING METHODS

A total of 10 023 adult cows from 323 dip tanks (DT) participated in the survey. A two stage cluster design approach was used. 30 DT were randomly selected from each State Vet Area (SVA) in KZN and then 30 adult cows were randomly selected from these chosen DT. Blood (10ml) was collected in plain vacuum tubes from each animal using the coccygeal (tail) vein. Serum was then separated from the blood and used for testing. RBT and CFT were used to test the samples. All samples that tested positive on RBT were tested with CFT, the serological confirmatory test for BB. Any sample that had a test result of > 23 CFT I.U was deemed positive. A DT with one or more positive animals was considered positive. A standard questionnaire was given to each animal health technician (AHT) responsible for each selected DT to complete.

## RESULTS

The crude sero prevalence of BB for KZN communal cattle was 1.3%. The overall DT prevalence for KZN was 8.6%. The DT prevalence of BB in the north, central and south regions was 24.4%, 2.4% and 0% respectively. DT's in the north region were 24.4 times more likely to be sero positive for BB compared to DT's in the south region. DT that reported abortions were 9.1 times more likely to be sero positive compared to those that did not have animals that had aborted.

## DISCUSSION AND CONCLUSION

The results of this study are in agreement with what previous studies have reported on the prevalence of BB in communal cattle in KZN. Any measures to control BB should be incorporated into the current FMD control measures as FMD is currently high on the priorities of state veterinary authorities in KZN. More attention needs to be paid to the control BB if communal farmers are going to benefit from other initiatives such as improving nutrition for their cattle. As a starting point, increased vaccination of susceptible animals should be undertaken as a matter of urgency. Since BB is a major zoonosis, further studies should be undertaken to measure the prevalence or incidence of BB in humans in areas with high BB prevalence. Such an approach will make it easier to convince policy makers to fund the control program if humans are shown to be affected.

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## RISK FACTORS OF DENGUE FEVER IN LAHORE, PAKISTAN

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### ABSTRACT

Dengue fever is a fatal mosquito borne viral disease in humans with serious public health concern in subtropical and tropical countries. There was an epidemic of Dengue fever in Lahore, Pakistan during 2011. An age and gender matched case control study with a main objective to study the risk factors of dengue fever in and around Lahore was designed. The cases were randomly selected from the patients of dengue fever admitted to the dengue isolation wards in a tertiary care hospital, whereas controls were the patients selected from the other wards of the same hospital. The data was collected through a structured questionnaire by interview. The statistical analysis was done by univariate analysis using Mantel-Haenszel odds ratios, chi square test and multivariate analysis using logistic regression in STATA® version 9.2.

Results showed that gender was a significant risk factor, possibly due to males being more active outside. Dengue cases were twice more likely to be males (OR= 2.16; 95% CI 1.35-3.43; p=0.001). The age significantly increased the risk of dengue fever (p<0.001); those aged between 16-45 years were more likely to be cases of dengue fever (OR= 3.19; 95% CI 1.43-7.1; p=0.004). The dengue cases were more likely in area with vegetation (OR= 3.03; 95% CI 1.81-5.06; p<0.001), swimming pools (OR= 2.52; 95% CI 1.4-3.84; p=0.001), stagnant water (OR= 3.13; 95% CI 1.87-5.25; p<0.001), vehicle service stations (OR= 2.52; 95% CI 1.53-4.16; p<0.001), tire shops (OR= 2.14; 95% CI 1.24-3.67; p=0.006) or garbage tanks (OR= 1.78; 95% CI 1.64-3.08; p<0.04). In conclusion the most important risk factors of dengue fever were the presence of vegetation (parks, trees, plants), swimming pools, stagnant water and ponds, vehicles service stations tire shops in the vicinity of the house. The males and those of age 16-45 were at greater risk of contracting dengue fever probably due to their outdoor activities.

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# MACRO-EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE IN THE CHAGAI DISTRICT, PAKISTAN

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## ABSTRACT

Livestock industry contributes 13.4% of the total GDP of Pakistan and is a major source of income for rural farmers, but is influenced by persistent outbreaks of diseases. Foot and mouth disease (FMD) causes an estimated loss of 6 million US\$ each year to the Pakistan livestock industry. The husbandry practices are different in different regions of Pakistan. The risk of FMD is very high in Chagai district due to trans-boundary animal movement between Pakistan, Iran and Afghanistan.

To evaluate risk factors and status of FMD in Chagai a macro-epidemiology study of FMD was conducted. Macro-epidemiology is the study of disease patterns with particular reference to social, political and cultural factors. A cross sectional study was designed to collect information about different factors like education and economic status of the farmers, veterinary services, import and export of livestock, vaccination and management practices. Information was collected from 323 farmers and/or livestock traders on a structured questionnaire. Data were statistically analysed: The chi-square ( $\chi^2$ ) test was applied to find the association between FMD and risk factors affecting macro-epidemiology of FMD in Chagai.

Results showed that education level and socioeconomic status of farmers, type of animals in the herd, open housing system, feeding methods in the case of farmers, grazing, lack of vaccination and veterinary services, nomadic movement, illegal import and export of live animals were ( $p < 0.000$ ) significantly associated with the occurrence of FMD in Chagai Pakistan. Vaccination was not practiced by 97% farmers. Other management practices like quarantine (no, 100%), isolation of sick animals from the herd (no, 100%), feeding of young animals milk of the sick mothers (yes, 100%) and proper disposal of the dead animals (no, 100%) were factors which were not analysed due to zero cell values. Trans-boundary movement of livestock to and from Afghanistan and Iran can aggravate the disease situation.

In conclusion, regular vaccination of livestock, provision of veterinary services, control of illegal trading of animals and awareness/training of farmers about better management of herds can help to improve the health status in context of FMD in Chagai. A macro-epidemiological study of FMD at national level is needed which may enhance the utility of quantitative risk assessment, by increasing risk communication and understanding among policy-makers and stakeholders from public and private sectors.

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# **CONTINUING EDUCATION PRESENTATION: ANIMAL HEALTH DECISION MAKING IN A ONE HEALTH CONTEXT**

Pfeiffer, D<sup>1</sup>

## **SUMMARY**

No summary available at time of publication.

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# DEVELOPING A MULTIPLE CRITERIA DECISION ANALYSIS TOOL TO ASSESS THE CONTROL OF FOOT-AND-MOUTH DISEASE IN SOUTH AFRICA

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## ABSTRACT

In 2012, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation of the United Nations (FAO) committed to a global Foot-and-mouth disease (FMD) control strategy. However, the FMD situation in southern African is different from elsewhere on the globe. The region hosts its own serotypes of the virus (the South African Territories (SAT) types 1, 2 and 3) and these serotypes are endemic in the wild buffalo populations, from which they are regularly transmitted to domestic cattle. Sub-Saharan Africa also has a wide range of livestock production systems and the priorities with regard to production goals and disease control differ widely. Eradication of FMD in southern Africa is consequently expected to be nearly impossible, by those familiar with the southern African situation.

Multiple criteria decision analysis (MCDA) is a tool used to assess a variety of options available to achieve an objective. The incorporation of multiple criteria and views from multiple stakeholders provides a method to identify important factors that need to consider when making a particular decision. This project aims to perform a multiple criteria decision analysis of the possible control methods to gain an understanding of the technical, economic and socio-political issues related to the prevention of FMD virus infection of domestic cattle in the free zone of South Africa.

Experts and stakeholders (including state veterinarians, commercial cattle farmers, game reserve representatives, veterinary staff in the Kruger National Park and representatives of the Department of Agriculture, Forestry and Fisheries in South Africa) will be invited to answer an online survey. A participatory epidemiology approach will be used to interview communal cattle farmers from eleven dip tanks and groups of state animal health technicians in the FMD protection zone with vaccination on the border of the Kruger National Park. Potential control options will be scored by participants according to a set of technical, economic and social criteria. Criteria will be weighted according to expert and stakeholder perceptions of their importance. The scores and weights will be combined to rank the control methods and scores and weights obtained from different stakeholder groups will be compared. A sensitivity analysis will be performed to explore the effects of varying inputs (scores and weights) on the final scores and rankings of the control options.

The project will produce a ranking of FMD control methods for use in South Africa and a comparison of stakeholders' views of the efficacy and viability of each. Furthermore, the effect of different stakeholders' viewpoints on the acceptability of FMD control measures and the extent to which stakeholders are likely to cooperate in enforcing the control measures will be estimated.

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# EPIDEMIOLOGY OF WEST NILE VIRUS AND ENDEMIC ARBOVIRUSES IN NEUROLOGICAL DISEASE IN SOUTH AFRICA: A ONE HEALTH APPROACH

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## BACKGROUND

West Nile virus (WNV) is endemic to South Africa but underreported as a cause of neurological disease. To investigate this and identify other arboviruses with zoonotic potential, a One Health approach was used since 2008 to trace virus activity, define seasonality, molecular epidemiology, disease potential and vectors involved.

## METHODS

Country-wide surveillance for acute neurological disease/fevers in horses was initiated in 2008. From 2010 postmortem specimens from fatal neurological cases in wildlife and livestock were also accepted. Mosquito vectors were trapped monthly since 2012 in 4 sites in Gauteng and Limpopo where horse and wildlife cases occurred. Specimens from humans with acute neurological disease were collected at 3 government hospitals in Pretoria (Gauteng) in 2008-2009 and again since 2012. Sera from 127 healthy veterinarians from across the country were screened for WNV and Shunivirus (2012). Acute specimens were screened by Flavi- and alphavirus genus specific PCRs followed by specific realtime-PCRs for WNV, Wesselsbron, Sindbis, Middelburg, Shuni, equine encephalosis (EEV); WNV IgM ELISA and positive cases sequenced. Neutralization assays was used to define sero-prevalence.

## RESULTS

WNV was detected annually in 1-17% of 609 horses (91% neurological and 36% fatal) (2008-2013). All PCR positive cases were lineage 2 except one fatal lineage 1 case. Other viruses identified by RTPCR in horses with neurological disease include Middelburg(7%) Sindbisvirus(1%), Shunivirus(1.7%), Equine encephalosis(9%) and Wesselsbronvirus(0.3%). 15/169 brain or spinal cord specimens from other species tested positive for Sindbis (2); Shunivirus (4), WNV(2); Middelburg (6) and EEV(1) representing 5/23 rhinoceros, 3/15 warthog, 1/28 Cape Buffalo, 1/6 crocodiles, 2/2 giraffe and 2/37 bovine. Cases were distributed across the country from February-June. Mosquito abundance peaked in February-April and is currently being screened. In hospitalized humans with neurological diseases, WNV was confirmed in 3.6% of 206 cases while 17% had neutralizing antibodies (2008-2009). WNV (7.9%) and Shunivirus(4%) antibodies were detected in 127 healthy veterinarians across the country in 2012. Serological screening for the other viruses is in process.

## CONCLUSION

WNV lineage 2 was associated with severe disease in horses and humans in South Africa, circulating across the country and predominated in late summer. Other endemic arboviruses with zoonotic potential contribute to neurological disease in several animal species and should be investigated in humans.

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# VACCINE MATCHING IN FMD CONTROL: A REVIEW

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## ABSTRACT

Foot-and-mouth-disease (FMD) is a highly contagious transboundary animal disease (TAD), which affects cloven-hoofed animals and reduces productivity of livestock in many countries. The disease is caused by FMD virus (FMDV) that belongs to the genus *Aphthovirus*, family *Picornaviridae*. There are seven serotypes of FMDV: O, A, C, Asia 1 and South African Territories (SAT) 1, 2 and 3 with multiple subtypes within each serotype. FMDV is a highly variable RNA virus and in general there is little or no cross-protection between serotypes and even between different strains of the same serotype.

Vaccination is one of the most important approaches in FMD prevention and control. The effect of vaccination mainly relies on the quality and suitability of the chosen vaccine. Although the quality of the vaccine used is the most important factor for the success of the vaccination programme, an antigenic match between the FMDV vaccine and the outbreak virus strain is also considered vital for the effectiveness of the vaccination. Vaccine matching is mainly applied for two main purposes; either to choose the most effective vaccine for use in particular circumstances, or to monitor on an on-going basis the suitability of vaccines maintained in vaccine antigen reserves. The selection of FMDV strain for matching is based on indirect serological methods, sequence data or alternatively on the calculation of the relatedness between the fields isolate and available vaccine strains using in-vivo challenge tests. Appropriate vaccine strain selection is an important element in the application of vaccination programmes in FMD control in affected regions.

In view of the importance of FMD vaccine matching to the success of FMD control in livestock, this study intends to highlight the direct comparative matching tests in-vivo cross protection methods including: (i) 50% protective dose ( $PD_{50}$ ), (ii) Protection against Podal Generalization (PPG) and (iii) Expected Probability of Protection (EPP). In addition indirect vaccine matching tests based on serological data which include: (i) Virus neutralization test (VNT), (ii) Compliment fixation test (CFT) and (iii) Liquid Phase Blocking ELISA will also be studied, while genetic characterization and antigenic profiling by CFT, ELISA or sequence analysis of the VP1 gene which can also show the emergence of new strains for which vaccine matching may be required will also be studied.

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