South African Association of Veterinary Technologists

The following are abstracts of papers and posters presented at the 'Back to Basics Congress' of the South African Association of Veterinary Technologists (SAAVT), 15–16 September 2009, Batter Boys, Pretoria, South Africa.

Oral presentations

A novel rotavirus from South African pigs is related to adult rotaviruses J19 from China and B219 from Bangladesh

I Peenze[a], A C Potgieter[b] and A D Steele[a,c]

[a]*Department of Virology and MRC Diarrhoeal Pathogens Research Unit, University of Limpopo: Medunsa Campus, South Africa*
E-mail: inap@ul.ac.za

[b]*Department of Virology, Onderstepoort Veterinary Institute, 0110 South Africa*

[c]*PATH, Seattle, USA*

Rotaviruses (RVs) are classified into 7 Serogroups (A–G). Group A RVs are the major cause of severe viral gastroenteritis in the young of humans and domestic mammalian and avian species. Groups B and C RVs are detected in both humans and animals, while Groups D to G are confined to mammals and birds. Traditionally, rotavirus Group A infections were more frequently detected in children under 5 years and very prevalent in children under 2 years. However, in 1997 an outbreak of gastroenteritis in adults in China was caused by rotavirus strains different from those in Groups A, B and C. The Chinese strain is similar to the novel adult rotavirus strain from Bangladesh called B219. Rotavirus particles consist of 3 protein layers; a) outer-viral capsid containing 2 viral proteins (VP4 and VP7, b) the inner capsid, VP6 protein, and c) a core capsid protein, VP2. The genome of 11 distinct segments of double-stranded (ds) RNA can be separated using polyacrylamide gel electrophoresis (PAGE) and segments are separated based on size and charge. Each rotavirus serogroup possesses a distinct PAGE migration pattern. The aim of this study was to characterize Group A and non-Group A RVs at a molecular level, using stool samples collected from pig farms in the provinces of Gauteng, North West Province, Free State and Mpumalanga in South Africa. All stool samples were screened for Group A RV antigen using a commercially available Rotavirus IDEIA kit (Oxoid, Ely, UK) according to the manufacturer's instructions. PAGE analysis of genomic dsRNA was performed on all ELISA-positive and -negative stool samples to identify serogroups. RV dsRNA was extracted from a 10 % stool sample solution using a TRIzol-phenol-chloroform method and precipitated with absolute ethanol. The PAGE gels were stained with silver nitrate to visualise the dsRNA segments. A sequence-independent genome amplification method was used to amplify the complete segmented genome from selected specimens. The cDNA of all genomes was sequenced using the ultra-deep DNA sequencing method (454/Roche GSFLX Sequencer, Inqaba Biotechnical Industries (Pty) Ltd). Rotavirus Group A could be detected in 44 % and 37 % of piglets during 2007 and 2008 respectively. Among the 9 Serogroup B RVs identified, 1 Group B sample (now termed Pig-N) revealed differences in PAGE migration patterns of the 11 gene segments compared to other Group B samples. Sequence homology and phylogenetic analysis of several genes of Pig-N clustered with the novel Group B-like adult rotavirus strains J19, from China and B219 from Bangladesh. The results demonstrate that other RV serogroups circulate in the pig environment which could potentially either reassort with human rotavirus strains to produce new strains or are capable of causing diarrhoeal disease in naïve populations. This research suggests that the novel Group B rotavirus detected in pigs may be related to the rare human adult Group B RVs from China and Bangladesh, and that the viruses from those outbreaks may have been of animal origin. Furthermore, these results highlight the importance of improved technologies for genome amplification and ultra-deep sequencing of rotavirus genomes. These techniques bypass the problems associated with primer-binding selection of specific genes and in the sequencing of large gene segments. Furthermore, there is a need to investigate non-Group A RVs circulating in domestic animal herds in South Africa to determine the significance of these strains in potential outbreaks.

A survey of trout farms in the Western Cape to establish the occurrence of *Flavobacterium* spp. in rainbow trout (*Oncorhynchus mykiss*)

B M Peyrot

Western Cape Provincial Veterinary Laboratory, Private Bag X5020, Stellenbosch, 7599 South Africa
E-mail: belindap@elsenburg.com

The Skills Development and Training Program of the Aquaculture Institute of South Africa (AISA) arranged the visit of a fish health specialist from Switzerland to various trout-producing farms in the Western Cape in February 2009. One of the aims of his visit was to do sampling to determine the occurrence and distribution of *Flavobacterium* spp. and *Aeromonas* spp. in the trout. Samples from the organs, skin and gills of trout on 7 farms were taken and inoculated onto bacterial culture media on the farms. Two types of media (Anacker and Ordal's agar and Columbia blood agar) were used for culturing. Incubation was carried out at 14–18 °C and at 25 °C. Various *Aeromonas* spp. from 4 farms and a *Flavobacterium* sp. from 1 farm were isolated. Phenotypical identifications of the *Aeromonas* spp. were performed using biochemical tests in conventional carbohydrate media as well as a commercial biochemical identification system. The *Flavobacterium* was presumptively identified on the basis of colony morphology on the selective medium, together with direct microscopy using Gram's stain. Although these bacteria are well-documented trout pathogens, this is the first time, to the knowledge of current staff, that this laboratory was asked to participate in this kind of survey for *Flavobacterium* spp. The media that were used appeared to adequately support the growth of both the control *Flavobacterium* isolate and the field isolate.

Introduction to the Food Safety Laboratory: activities at the Western Cape Provincial Veterinary Laboratory, Stellenbosch

T Potgieter

Food Safety Section, Veterinary Provincial Veterinary Laboratory, Stellenbosch, 7599 South Africa
E-mail: theop@elsenburg.com

The Food Safety section at the Western Cape Provincial Veterinary Laboratory (WCPVL) was established 14 years ago and was part of the Meat Inspection Services since the early 1980s. The main function of the laboratory was to monitor the hygiene of local abattoirs and cold storage facilities in the Western Cape and at a later stage, meat imports from European countries. Currently the laboratory monitors an average of 160 containers of imported frozen poultry meat per month. Samples are collected by health officials from the National Department of Agriculture (NDA) and forwarded to the WCPVL in Stellenbosch. These products are required to adhere to the microbiological standards set by the European Union specified on importation certificates and our laboratory conducts quantitative tests to ensure that these products comply with recommended standards. These test procedures are all based on methods used by the South African Bureau of Standards (SABS) and entail bacteriological monitoring of feed and water samples and locally produced meat, and screening for microbial residues. The following tests are conducted on the food/feed: Total plate counts, Enterobacteriacea, coliforms, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas* sp. counts, and *Salmonella* and *Listeria* detection. Water analysis tests consist of total plate counts, faecal coliform and *Escherichia coli* counts, faecal *Streptococcus* counts and, to a lesser extent, Clostridia counts. The implementation of the Nordic Committee on Food Analysis (NMKL) procedure of 2008 (measurement of uncertainty in quantitative microbiological examination of foods) has resulted in an increase in workload, but yields more reliable results. This laboratory also participates in different inter-laboratory testing schemes

DIptera of veterinary importance – focusing on *Culicoides* biting midges

K Labuschagne

Entomology, ARC-Onderstepoort Veterinary Institute, PO Box X05, Onderstepoort, 0110 South Africa
E-mail: labuschagnek@arc.agric.za

Culicoides biting midges are important worldwide in disease transmission. These small biting flies transmit a number of protozoa, nematodes and viruses to humans and livestock. Not all *Culicoides* species are able to act as vectors of the pathogens that may be transmitted. In South Africa, African horsesickness (AHS), bluetongue (BT) and equine encephalosis (EE) are 3 economically important virus diseases that may be transmitted. It is thus of vital importance to be able to identify these insects correctly. Unfortunately, in practice, these insects are often confused with other small biting flies such as *Simulium*, *Leptoconops*, *Forcipomyia*, *Lasiohelia* and *Phlebotomus* species. There are some important differences between all of these insects, such as body and wing shape, colouration, diseases transmitted and habits. In appearance some of these insects differ greatly from each other, while others are similar. Some are able to transmit diseases while others are not. A short description of each insect group will be given along with their importance in disease transmission.

Optimization of the Montanide™ ISA 206 adjuvanted foot-and-mouth disease vaccines containing the South African Territories (SAT) serotypes

F Peta, M Cloete[a,b] and L van Staden[a]

[a]*Transboundary Animal Diseases Programme, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa*
E-mail: petaF@arc.agric.za

[b]*Crop Protection Division, ARC-Roodeplaat Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria, 0001 South Africa*

Foot-and-mouth disease (FMD) is an economically important disease of cloven-hoofed animals that is primarily controlled by vaccination of susceptible animals and movement control of animals and animal-derived products in South Africa as prescribed by the Animal Diseases Act 35 of 1984. The Seppic Montanide™ ISA 206 B oil vaccine induces a protective immune response in all susceptible animals when used shortly after formulation and, compared to the aqueous ALSA vaccine, has been shown to be the most suitable oil adjuvant to use with the South African Territories (SAT) serotypes of FMD. However, this vaccine needed to be optimized to ensure sustained vaccine stability for at least 12 months of storage at 4 °C. A stable vaccine is indicated by: maintenance of pH levels ≥7.0, a water-in-oil-in-water (W/O/W) emulsion type and consistent emulsion size. Different buffers, buffer concentrations, buffer:antigen ratios and storage temperatures to be used in this oil vaccine were tested to determine the most suitable parameters which would ensure vaccine stability over at least 12 months of storage. The effects of saponin (an immuno-stimulant), if any, on the vaccines' pH were determined. A combination of Tris and KCl buffers (concentrations are trade secrets) with 10 % antigen of the aqueous phase of vaccine volume, proved to be the most suitable buffer for oil vaccines as it was able to maintain pH levels ≥7.0, a W/O/W emulsion type and consistent emulsion size over 24 months at 4 °C. It was closely comparable to the Seppic recommended KH_2PO_4 buffer which was used as a control. Saponin does not negatively affect the vaccine's buffer as no significant drop in vaccine pH or changes in vaccine emulsion type or size were noticed over the testing period. Further research is required to determine the optimized ISA 206 B efficacy in cattle.

Detection of *Campylobacter fetus* in bovine preputial scrapings using PCR and culture assays

T Schmidt, E H Venter and J A Picard

Allerton Provincial Veterinary Laboratory, Private Bag X2, Cascades, 3202 South Africa
E-mail: tracy.schmidt@kzndae.gov.za

The traditional method for the diagnosis of campylobacteriosis is the culture and identification of the causative organism, *Campylobacter fetus* subsp. *venerealis* (Cfv). This approach is considered to be relatively insensitive owing to the fragility of the bacteria, their specific nutritional and atmospheric requirements, and their being easily overgrown by commensal bacteria. With the rapid advances which have been made in the molecular field, polymerase chain reaction (PCR) assays have become more robust and cost-effective, making them more feasible for routine use in the diagnostic laboratory. The aim of this study was to evaluate a previously published *C. fetus*-specific PCR to detect *C. fetus* directly in diagnostic specimens, specifically bovine preputial specimens. The specificity of the assay was established by testing *C. fetus* reference and field isolates in addition to a collection of other *Campylobacter* species and organisms which may be encountered in the genital tract of cattle. All *C. fetus* isolates tested yield a single PCR amplicon of approximately 750 bp. No amplicons were generated when any of the other non-*C. fetus* isolates were tested. The sensitivity of the assay was determined using spiked Weybridge medium. A detection limit of 615 Cfv/ml Weybridge medium (or 6.15 cells equivalents per PCR assay) was obtained. Once the detection limit had been established, the influence of potential PCR inhibitors (faeces, urine, semen and blood) on the sensitivity of the assay was evaluated. Faeces were identified as a potent inhibitor of the PCR assay and contamination of specimens with as little as 1 % (w/v) faeces significantly reduced the sensitivity of the assay. Concentrations of up to 50 % (v/v) of blood, urine and semen were shown to have no effect on the sensitivity of the assay. Preputial specimens, collected in Weybridge medium, were subsequently pooled and spiked, and used to establish the sensitivity of both the PCR and culture methods as well as determine the influence of time on the sensitivity of the assays. Testing was carried out in triplicate on samples collected from different herds which were ascertained to be free of Cfv based on the use of specific selection criteria. The detection limit of the culture method was found to be better than that achieved using PCR only immediately after the samples were spiked. The detection limit of the culture method decreased with time while the detection limit of the PCR assay remained unchanged for up to 72 hours post-inoculation. Ensuing field evaluation involved the testing of 212 clinical samples using both the culture method and the optimized PCR assay. Of the samples tested 4.2 % were found to be positive using the PCR assay, whilst only 3.8 % were found to be positive by culture. Based upon this evaluation the analytical specificity of the PCR assay was calculated to be 99 % and the analytical sensitivity 85.7 %.The PCR assay was shown to be both specific and sensitive, and overall test performance compared rather favourably with the culture method. The multiple advantages offered by PCR make the assay an attractive alternative to conventional methods available for use in diagnostic laboratories.

The role and influence of government control programmes for rabies in KwaZulu-Natal

K Le Roux

Epidemiology Section, Allerton Regional Veterinary Laboratory, South African Veterinary Services KwaZulu-Natal, Private Bag X2, Cascades, 3202 South Africa
E-mail: k.leroud@kzndae.gov.za

The observation that the level of canine rabies in a country or province is directly related to the immunity level of its canine population should focus our attention on the factors that influence the immunity. The 6 major outbreaks of rabies in KZN over the past 42 years can all be directly linked to government control programmes and their successes and failures. Other factors such as virus evolution and ecology of the host species have, however, also played a role, albeit very small, in the progress of the disease. Molecular biology of the virus showed that the canine biotype still dominates, with no evidence of a wildlife host, in KZN. Many of the original factors that influenced the arrival and establishment of the virus in the canine population remain present. New evidence of a growing stray dog population owing to socioeconomic factors such as the AIDS epidemic and rising poverty in the province, will complicate future control strategies. Analysis of the successes and failures over the past 42 years have highlighted many key issues in canine rabies control. These include sustainability, importance of political

collaboration, constant improvements in control strategies, financial input and the need for someone to drive the process. Rabies in KZN has been shown to be completely controllable and, with the improvements in control measures and international support, canine rabies could be eliminated from the province in the foreseeable future.

Monitoring genetic variability of foot-and-mouth disease virus strains circulating in the African buffalo (*Syncerus caffer*) population in the Kruger National Park

B Botha[a], R M Dwarka[a], N T E Mtshali[a] and R Bengis[b]

[a]*ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme, Private Bag X05, Onderstepoort, 0110 South Africa*
E-mail: bothab@arc.agric.za

[b]*Division of Veterinary Services, PO Box 12, Skukuza, 1350 South Africa*

Foot-and-mouth disease (FMD) is a highly contagious, economically devastating viral disease of cloven-hoofed animals, affecting both livestock and game. The virus is serologically heterogeneous with 7 serotypes occurring worldwide, namely serotypes A, O, C, South African Territories (SAT)-1, SAT-2, and SAT-3, and Asia 1. The Kruger National Park (KNP) is the only region in South Africa where FMD is endemically present, the remainder of the country being recognised as free of the disease. The African buffalo (*Syncerus caffer*) population in the KNP is persistently infected and acts as a maintenance host of the SAT types of FMD viruses, and represents a potential source of infection to other susceptible species. FMD infections in buffalo are generally subclinical, and a long-term carrier state develops. Transmission from carrier buffalo to other species has been demonstrated both under experimental and natural conditions, thereby requiring the National and Provincial Directorates of Veterinary Services to focus on establishing and maintaining control measures to prevent the spread of the disease to adjacent domestic animal populations. A thorough understanding of the behaviour of SAT viruses in wildlife and transmission of FMD from wildlife to livestock is critical in addressing FMD control in the face of increasing integrated land-use. Sera and pharyngeal scrapings from various buffalo herds throughout the KNP are submitted on a regular basis by State Veterinary Services (KNP) to the Diagnostic Section of the Transboundary Animal Diseases Programme (TADP) at ARC-Onderstepoort Veterinary Institute (OVI). Samples are processed and analysed in order to genetically and antigenically characterise FMD virus isolates circulating within the buffalo populations. Monitoring of FMD field isolates is crucial in ensuring the suitability of vaccines currently used in the endemic control zone bordering the KNP.

A retrospective serological analysis of the spatio-temporal activity of Rift Valley fever virus infection in the Kruger National Park

J Joubert[a,b] and R T F Bernard[a]

[a]*Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa*

[b]*Veterinary Wildlife Services, Kruger National Park, South African National Parks, South Africa*
E-mail: jenny@sanparks.org

In the Kruger National Park (KNP) there is the possibility of Rift Valley fever virus (RVFV) being transmitted across the fence from buffalo to neighbouring community cattle. Thus there is a need to understand the spatial and temporal occurrence of RVFV in the KNP and how this is related to climate. The aim of the study was to establish the prevalence of RVFV in space and time throughout KNP by examining the biotic (buffalo age and sex) and abiotic (rainfall) factors affecting the prevalence and distribution of the virus. Serum samples collected from 1487 buffaloes sampled during BTB surveys in KNP in 1996, 1998, 1999, 2005, 2006 and 2007 were analysed by means of an indirect enzyme linked immonosorbent assay (I-ELISA) technique based on the recombinant nucleocapsid protein (recNp) for RVFV antibodies. Results indicate that RVFV is endemic to the KNP. We found no significant relationship between high rainfall and RVFV prevalence. There was, however, a significant change in prevalence over time. Analyses showed that there was a significant difference between RVFV positive females compared to males and also between adult females compared to adult males. GIS maps were created using GPS coordinates showing positive and negative herds for each year buffalo were sampled.

Determining baseline blood chemistry values of free-ranging African buffaloes (*Syncerus caffer*) and the effect of bovine tuberculosis

J Joubert[a,b] and R T F Bernard[a]

[a]*Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa*

[b]*Veterinary Wildlife Services, Kruger National Park, South African National Parks, South Africa*
E-mail: jenny@sanparks.org

Baseline blood chemistry values for African buffaloes (*Syncerus caffer*) in the Kruger National Park were analysed statistically to ascertain their usefulness in detecting bovine tuberculosis (BTB) at an early stage and used as a diagnostic tool. An ANOVA was used to evaluate the effects of age, sex and BTB status on blood chemistry values. Parameters tested included chloride (Cl), creatinine, potassium (K), urea, sodium (Na), calcium (Ca), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and immunoglobulins. Age affected the concentrations of K, ALT, AST, creatinine and urea. The results showed that BTB positive adult and/or subadult buffaloes had significantly higher K, creatinine and Cl concentrations than BTB negative animals. BTB positive juveniles had significantly lower chloride and AST concentrations than BTB negative animals, and BTB positive buffaloes of all age groups had significantly lower urea concentrations than BTB negative buffaloes. The BTB status of buffaloes had no effect on the ALT, IgG, Na and Ca concentrations. Baseline blood chemistry values are not a useful tool for early indication of BTB.

Epidemiology of anthrax in the Kruger National Park

A Dekker

PO Box 12, Skukuza, 1350 South Africa
E-mail: atd@nda.agric.za

Anthrax has been known since recorded history. Descriptions of anthrax are in the biblical *Book of Exodus,* where the disease was referred to in the 5th and 6th plagues of Egypt (1491 BC). Anthrax is caused by a bacterium, *Bacillus anthracis.* All mammals are believed to be susceptible to anthrax, especially ruminants. Three forms are described in animals: peracute (apoplectic), acute, and subacute to chronic. Anthrax has been a part of the Kruger National Park ecosystem for as long as there has been game in the region. In certain areas it can be described as indigenous (endemic) because of the frequency of outbreaks, or presence of viable spores in the soil or bones. The first described and positively diagnosed outbreak in the Kruger National Park was in 1959 in the Shingwedzi area. This outbreak later spread to the Punda Maria ranger section and neighbouring Pafuri area. The latter is probably the area with the highest frequency of outbreaks in the Park. This can be ascribed to the fact that it was discovered to be the area where the most anthrax spores have been found in the soil and old bones (carbon-dated bones of 250 years have been found to harbour viable anthrax spores). In the last 20 years there have been 2 major outbreaks in the Park. The first one in 1992 started on the Letaba section and then rapidly spread to neighbouring sections. All species were affected with kudu being the prime victim. Thereafter outbreaks in 1993 and 1999 took place in the Kingfisherspruit section; once again kudu were the prime victims. Outbreaks of anthrax in the Park can be linked to rainfall: most outbreaks have occurred in winter after a dry or low rainfall summer. The opposite usually happens if it starts to rain during an outbreak, as the number of cases declines rapidly until only isolated cases are recorded for a time and then none. In the park there are 3 cycles that play a major role in the maintenance and spread of the disease namely, vultures, blowflies/kudu or scavengers. The disease is diagnosed using 1 of the following methods: smears made from blood or exudates, bacterial cultures prepared from any body part such as skin, muscle, bone and soil in the vicinity of the carcass, control of anthrax in wildlife: It is impractical to implement any

control measures in the Kruger National Park for wildlife, as is done for domestic animals, such as immunization and treatment, except in areas where biodiversity is threatened. Anthrax carcasses are disposed of by burning or burying after covering with lime. Continuous surveillance is of great importance to locate and monitor all outbreaks. Blood smears should be collected from all carcasses including animals killed by carnivores. All unnatural deaths should be reported. Tourist awareness is also important in monitoring abnormal vulture activity. The zoonotic potential of anthrax should be emphasized. Anthrax can be contracted by 3 different methods: inhalation, cutaneous and ingestion.

Poster abstracts

Detection of feline coronavirus in cheetahs using real-time polymerase chain reaction

A-M Bosman[a], M van Vuuren[a], J S Peck[b], J M Bernard[b] and M Kennedy[b]

[a]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, 0110 South Africa*
E-mail: bossie.bosman@up.ac.za

[b]*Department of Comparative Medicine, University of Tennessee, Knoxville, Knoxville, TN, USA*

Feline coronavirus infection is a contagious viral disease that can be a serious pathogen of members of the family Felidae and is transmitted *via* the gastrointestinal and respiratory tracts. The viral genome consists of a long positive stranded RNA of 27–31 kilobase pairs (kb). It is believed that the 7b open reading frame (ORF) gene plays a role in the virulence of the virus and that the 7a ORF plays a role in the development of the disease. Genes 7a and 7b were used as target genes in the reverse transcription real-time polymerase chain reaction (rt RT-PCR) assay to study the prevalence of coronavirus in captive populations of cheetahs. Faecal specimens were collected from healthy cheetahs from different conservation centres and samples were also obtained during necropsy from cheetahs following various causes of death. RNA was extracted using the QIAamp® Viral RNA mini kit (Qiagen, Southern Cross Biotechnologies) as protocol. A One-step quantitative rtRT-PCR was performed using Superscript III Platinum, and the One-Step qRT-PCR System according to the manufacturer's recommendations. Primers to a conserved region of the 7b gene of coronavirus as well as a probe (TaqMan, Applied Biosystems) specific to an internal genetic region of the target were used. Only a small number of the samples tested positive and some of the specimens have late curves. One of these samples, 'Jake', was collected from a clinically sick cheetah. The other samples with late curves were collected from animals that did not have clinical disease. Late peaks require further investigation, especially the sample labelled 'Jake'. The low number of positive samples may be due to the fact that the probe used was not specific enough and that a new probe must be designed that targets a larger area in the gene of interest. More sequencing results are therefore required.

What is in a name? – *Salmonella* nomenclature and serotyping

M Maartens

Deltamune (Pty) Ltd, PO Box 14167, Lyttelton, 1040 South Africa
E-mail: michele@deltamune.co.za

Salmonella nomenclature is very intricate and scientists use diverse systems to communicate with reference to this genus. There are currently 2579 serovars of *Salmonella* in the 2 species and 6 subspecies of the genus *Salmonella*. These are defined and maintained by the World Health Organization Collaborating Centre and are listed in the White-Kauffman-Le Minor scheme. Before the taxonomy of the genus *Salmonella* was established on a scientific basis, *S. enterica* subspecies were considered as subgenera and serovars were treated as species, for example *Salmonella typhimurium*. Molecular methods have shown that the genus *Salmonella* consists of only 2 species *S. enterica* and *S. bongori*. Kauffmann has led the nomenclature on the basis of serological identification of somatic (O) and flagellar (H) antigens, but historically O groups were first designated by letters and since there were not enough letters, it was necessary to continue with numbers 51 to 67. Along with the reclassification of the genus this has led to immense confusion between the scientist, health officials and the public. However, the nomenclature for *Salmonella* is still evolving and the debate will not be settled soon. We believe that clarity on the nomenclature established by the WHO collaborating centre is needed for the veterinary technologist reporting *Salmonella* serovars and this poster explains what is currently considered to be correct. As an example, should *Salmonella typhimurium* now be written as the very cumbersome *Salmonella enterica* subsp. *enterica* serovar Typhimurium, or *S. enterica* subsp. *enterica* serovar Typhimurium, or *Salmonella* ser. Typhimurium, or *S. enterica* serovar Typhimurium, or *S.* Typhimurium?

Echinococcus survey in Eastern Cape Province, South Africa

C Boshoff

Tshwane University of Technology, Private Bag X680, Pretoria, 0001 South Africa
E-mail: boshoffci@tut.ac.za

Echinococcosis is a zoonotic disease caused by the parasitic flatworms (Cestodes) of the genus *Echinococcus*. The disease is of worldwide importance because of its potential economic impact. *Echinococcus granulosus* is known to occur in South Africa but an accurate assessment of its incidence is difficult owing to the lack of recent and more comprehensive data. The adult worm resides in the digestive tract of canines and felines which act as definite host. In the intermediate hosts, which include humans, hydatid cysts develop in internal organs. The potential for domestic transmission of *E. granulosus* is highest in poor countries where the level of education is low, humans and domestic animals live close together, and home slaughtering practices are common. South Africa is regarded as a developing country with a large portion of the population living in rural areas. Rural populations are still to a large extent dependent on animals for food, transport and cultivating the land. Based on the number of cases of echinococcosis reported to the Department of Animal Health, Veterinary Services, SA, rural areas of Eastern Cape were selected in which to do a 4-month survey of the prevalence of the parasite in domestic animals to establish whether this disease is still prevalent in South Africa. Specimens were collected during home-slaughtering practices. Cysts were found in the livers and lungs of goats and sheep from different rural communities. This indicates that echinococcosis is still prevalent in the Eastern Cape Province, South Africa. It's extent, distribution and the genetic strain of the parasite, however, still need to be identified.

Validation and accreditation of the test method for total aflatoxins in feed samples using the enzyme-linked immuno-absorbent assay (ELISA)

G C H Ferreira, H van den Berg and D Naicker

Toxicology Section, Food, Feed and Veterinary Public Health, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa
E-mail: ferreiraa@arc.agric.za

Aflatoxins are secondary metabolites that are produced by certain strains of the moulds *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins cause cancer, liver damage and interference with reproductive efficiency. Chronic aflatoxicosis in adult ruminants can cause anorexia, drying and peeling of the skin on the muzzle, rectal prolapse, and abdominal edema. Aflatoxicosis has also been shown to cause decreased fertility, abortion, and lowered birth weights in sheep. Large doses of aflatoxins have been shown to produce hepatic necrosis in pigs. A competitive direct enzyme linked immunoabsorbent assay (ELISA) is used to determine the total aflatoxin in feed samples. In this type of ELISA, there is an inverse relationship between the signal obtained and the concentration of the analyte in the sample, due to the competition between the free analyte and the ligand-enzyme conjugate for the antibody coating the microplate, *i.e.* the more analyte the lower the signal. The validation process included quality control, intermediate checks, test performance of the method using reference materials, validating data of linearity and range ($r \geq 0.99$); recovery ($\geq 93.4\ \% + 7\ \%$(RSD)], precision

(≤11.9 % RSD), limit of detection (3 ppb), limit of quantification (5ppb) and accuracy. Accuracy was proven through proficiency testing (pass rate of 95 %) received from 2 international schemes (FAPAS and Neogen). An evaluation of measurement uncertainty (MU) is also expressed in a way that can be given to the client on request. It describes the range around a reported or experimental result in which the true value can be expected to lie within a defined level of probability. All these processes have been audited by SANAS according to ISO 17025 requirements and have been deemed acceptable for accreditation (granted in November 2008).

The road to laboratory accreditation according to ISO/IEC 17025: 2005 standard: 'General Requirements for the Competence of Testing and Calibration Laboratories'

B Cloete and N Moyo

Western Cape Provincial Veterinary Laboratory, Stellenbosch, 7599 South Africa
E-mail: bronwyncloete@gmail.com

ISO 17025: 2005 – 'General Requirements for the Competence of Calibration and Testing Laboratories' was published by the International Organization for Standardization (ISO) in December 1999 and revised in May 2005. It is the result of a joint partnership between the ISO and the International Electrotechnical Commission (IEC). It replaces ISO Guide 25 and EN 45001 and takes into consideration the operating experience gained since the previous standards were published. ISO 17025: 2005 was developed specifically to give guidance to laboratories on both quality management and the technical requirements for proper operation. This standard can be considered the technical complement to ISO 9000. Consequently, any organization that satisfies the requirements of ISO 17025: 2005 also meets the intent of ISO 9000 requirements; however, the reverse is not true. While the ISO 9000 requirements are generic and are intended to be applicable to any type of organization, the ISO 17025: 2005 requirements are specific to testing and calibration laboratories. This standard addresses issues such as: the technical competence of personnel, ethical behaviour of staff, use of well-defined test and calibration procedures, participation in proficiency testing, and contents of test reports and certificates. Another reason for development of the standard was to harmonize laboratory accreditation and acceptance of test data worldwide. All participating countries will be required to accept the test results performed by accredited members of the other countries. This poster aims to demonstrate the various stages a laboratory needs to embark on in order to attain ISO 17025 accreditation, and the potential challenges that could be faced during this process. Although it was developed for specific use at the Western Cape Provincial Laboratory, it also considers the situation of other provincial laboratories. Bearing in mind that laboratory test results are a significant component of the information used to make crucial decisions in veterinary medicine, it stands to reason that errors in laboratory tests can lead to serious misjudgements in health management. Quality assurance procedures in the laboratory minimize errors and provide confidence in the validity of laboratory test results. Ultimately, therefore, it can be said that the importance of Quality within the laboratory environment cannot be emphasized enough, especially in the ever-competitive world where Quality, as perceived by the customer (in the case of laboratories, the department, external veterinarians and farmers) will make the essential difference between the survival or not of any laboratory in the developing world.

Guide to sample collection and preparation as well as test selection for the rapid confirmatory diagnosis of Rift Valley fever virus infection in livestock

S J E Bosch and R D Last

Vetdiagnostix - Veterinary Pathology Services, PO Box 13624, Cascades, Pietermaritzburg, 3202 South Africa
E-mail: butchs@vodamail.co.za

Rift Valley fever (RVF) is a mosquito-transmitted disease of livestock and humans which has historically been associated with large epidemics of devastating disease throughout Africa and, more recently, the Arabian Peninsula. The recent 2009 outbreak in Kwazulu-Natal has highlighted the changing face of RVF in South Africa, alerting us to the probable emergence of enzootic RVF foci in the country. Against this background it is clear that there is a greater need to be more vigilant about the safe handling of samples. The aim of this study was to compile a list to improve on the rapid and safe diagnosis of the disease. Essential requirements for handling and processing RVF samples include wearing protective clothing and disinfecting all instruments, clothing and the necropsy area with antiviral solution on completion of the necropsy, and ensuring safe carcass disposal. The organs of choice for confirmation of the diagnosis are liver, lymph nodes, spleen, lung, kidney plus adrenal glands and placenta, fixed in 10 % buffered formalin. All samples must be processed in a bio-hazard cabinet. Effective control of RVF requires rapid response, warranting the selection of reliable rapid confirmatory diagnostic assays, to enable prompt implementation of control procedures. RVF is also a notifiable disease in South Africa and all positive results must be forwarded to the local State Veterinarian. All necessary steps must be taken to ensure rapid and safe diagnosis.

Determining the optimal growth conditions for South African Territories (SAT) type foot-and-mouth disease (FMD) strains in suspension culture.

K Malan, P Dolo, B Ngobeni, L Ikaneng, F Peta and J. van Heerden

Transboundary Diseases Programme, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa
E-mail: petaf@arc.agric.za

Foot-and-mouth disease (FMD) virus South African Territories (SAT) serotypes 1, 2 and 3 are endemic in African buffalo (*Syncerus caffer*) populations in the Kruger National Park (KNP) and adjoining game farms in the northeastern corner of South Africa (SA). This area, comprising the only FMD infected zone in SA, is separated by a buffer and surveillance zone from the rest of the country which is called an FMD-free zone. Vaccination of cattle occurs only in the buffer zone where ±55 000 animals are vaccinated twice annually with a trivalent vaccine adjuvanted with alhydrogel-saponin (AS). The current FMD vaccine contains 4 strains that are representative of all 3 SAT serotypes of FMD and were previously produced in a monolayer production plant (MPP). The production of FMD antigen in a suspension production plant (SPP) with a 600 litre capacity is currently in the developmental stage. Production yields can be improved by determining the optimal harvest time for each of the FMD vaccine strains in the SPP. Factors that contribute to the effective recovery of SAT virus in suspension cultures include the multiplicity of infection (m.o.i.), the cell entry pathway, time of harvest (TOH), and the stability of the virus and the antigen after inactivation. In this study different m.o.i. were investigated for optimal recovery of the SAT-type viruses in the suspension culture. The ability of the viruses to infect baby hamster kidney (BHK)-21 cells, the production cells and Chinese hamster ovary (CHO)-K1, non-natural host cells, provided information regarding the cell entry pathway of the vaccine strains. BHK-21 cells express both integrins, $\alpha_V\beta_6$, and heparin sulphate surface receptors that are required for FMD virus entry into the cells, whereas CHO-K1 cells only express heparin sulphate receptors on their cell surfaces. The results indicated that the optimal harvest time was between 13–19 h for SAT strains grown on small scale (500 mℓ) in suspension. The optimal TOH for each SAT strain was dependent on the m.o.i used for virus seeding. The BHK and CHO cell lines had varying susceptibilities to the viruses.

Optimisation of the production process is a continuous process and research information obtained can be implemented in future research projects to ensure a vaccine which will provide optimal protection in animals.

Culicoides species of South Africa (Diptera: Ceratopogonidae). The Imicola group

K Labuschagne

Entomology, PVVD, ARC-Onderstepoort Veterinary Institute, PO Box X05, Onderstepoort, 0110 South Africa
E-mail: labuschagnek@arc.agric.za

Culicoides biting midges transmit economically important viral

diseases such as African horse sickness (AHS), bluetongue (BT), equine encephalosis (EE) and epizootic haemorrhagic disease of deer (EHD). In South Africa, *C. imicola* and *C. bolitinos* are considered to be the primary vectors of these diseases. Both these species belong to the subgenus *Avaritia* and are members of the Imicola group. The Imicola group comprises at least 10 species worldwide, of which 6 occur in South Africa. The 6 species within the Imicola group in South Africa are *C. imicola, C. bolitinos, C. loxodontis, C. tuttifrutti, C. miombo* and *C.*sp.#107 (a species not yet described). Distinguishing characteristics of the subgenus *Avaritia* and the Imicola group are discussed along with distribution maps and examples of the breeding site of each species are given. The role of each of these species in disease epidemiology is indicated.

Assigning *Campylobacter fetus* isolates to subspecies using genotyping and phenotyping assays

T Schmidt, E H Venter and J A Picard

Allerton Provincial Veterinary Laboratory, Private Bag X2, Cascades, 3202 South Africa
E-mail: tracy.schmidt@kzndae.gov.za

Currently, the only test recommended by the Office International des Epizooties (OIE) for assigning *Campylobacter fetus* isolates to subspecies, is tolerance to 1 % glycine. Doubts over the reliability of this test have led to alternative or supplementary tests being sought. Within the context of this investigation a collection of 40 South African field isolates were identified to subspecies using a previously described multiplex PCR as well as the traditional 1 % glycine tolerance phenotyping test. Additionally, other phenotyping tests (selenite reduction, growth at 42 °C and susceptibility to metronidazole and cefoperazone) were evaluated to determine their suitability for use as an aid in assigning *C. fetus* isolates to subspecies. None of the *C. fetus* field isolates tested yielded a subspecies-specific amplicon when tested using the multiplex PCR assay, suggesting that all of the isolates were *Campylobacter fetus* subsp. *fetus* (Cff). Based on the glycine tolerance test, however, only 6 isolates were identified as Cff, whilst the rest were classified as Cfv. The results of the 'sensitive' hydrogen sulphide test indicated that the Cfv isolates were specifically Cfv biovar intermedius. The multiplex PCR failed to differentiate this specific 'group' of isolates, resulting in the incorrect subspecies identification of isolates. Owing to the seemingly large proportion of Cfv biovar intermedius isolates in South Africa and the unpredictable results obtained with this group of organisms when using the multiplex PCR, the assay is deemed unsatisfactory for assigning SA field isolates to subspecies. None of the other phenotyping tests evaluated showed 100 % correlation with subspecies differentiation based on glycine tolerance. The selenite reduction test, which has been used with varying degrees of success by different laboratories, was found to be unreliable with variable results being obtained between replicate tests. In order to resolve the problems experienced in the laboratory with the assignment to subspecies of *C. fetus* isolates it appears that more specialized genotyping techniques are going to be needed. These techniques are largely beyond the resources of routine diagnostic laboratories, requiring technical expertise and expensive hardware. Thus for the time being the only option available to diagnostic laboratories is the glycine tolerance test. Owing to the importance of this test, it is recommended that the test be carried out in duplicate in order to eliminate the possibility of an aberrant, incorrect result being reported.