

Proceedings of the 11<sup>th</sup>  
annual congress of the  
Southern African Society  
for Veterinary  
Epidemiology and  
Preventive Medicine

14-16 August 2013



ISBN: 978-0-620-57567-6

## ACKNOWLEDGEMENTS

### CONTINUING EDUCATION PRESENTERS

Dr. Rebone Moerane  
Chair: Primary Animal Health Care  
Faculty of Veterinary Science  
University of Pretoria

Misheck Mulumba, PhD  
Project Coordinator and Regional Surveillance Network Expert SADC/ADB  
Strengthening Institutions for Risk Management of TADs Project

### SPONSORSHIPS

SASVEPM are grateful for the continued support of the sponsors of their annual congress and in this case specifically ONDERSTEPOORT BIOLOGICAL PRODUCTS for the support this year



*The views expressed in these proceedings are not necessarily those of the Executive Committee of SASVEPM. Presentations based on previously published work are included in title and author list only and for full detail on these articles please refer to the published work. Some work presented at the meeting was not available at the time of printing of this document and only the titles have been listed in these cases.*

# TABLE OF CONTENTS

CONTINUING EDUCATION PRESENTATION: PRIMARY ANIMAL HEALTH CARE CONCEPT .....	1
<i>Moerane, R<sup>1</sup></i>	
EVALUATION OF ELISA FOR DETECTION OF RABIES SPECIFIC ANTIBODIES IN DOMESTIC DOGS: A PRELIMINARY STUDY .....	2
<i>Ngoepe<sup>a*</sup>, E. &amp; Sabeta, C.<sup>a</sup></i>	
GENETIC ANALYSIS OF DOMESTIC AND WILDLIFE RABIES VIRUSES FROM THE NORTH-WEST (2012) DEMONSTRATE A COMMON RABIES CYCLE.....	7
<i>Sabeta, C.<sup>*</sup>, Phahladira, B., Miyen, J., Mohale, D., Ngoepe, E. &amp; Shumba, W.</i>	
EMERGENCE OF RABIES IN THE GAUTENG PROVINCE, SOUTH AFRICA: 2010-2011.....	9
<i>Sabeta, C.T., Weyer, J., Geertsma, P., Mohale, D., Miyen, J., Blumberg, L.H., Leman, P.A., Phahladira, B., Shumba, W., Walters, J. &amp; Paweska, J.T</i>	
RABIES STATUS IN KWAZULU NATAL .....	10
<i>Le Roux, K.<sup>1</sup></i>	
CONTINUING EDUCATION PRESENTATION: IMPACT OF TRAINING USING A STRUCTURED PRIMARY ANIMAL HEALTH CARE MODEL ON THE SKILLS OF RURAL SMALL SCALE FARMERS .....	11
<i>Moerane, R., <sup>*</sup>1 Terblanche, S.E., <sup>2</sup> Irons, P.C. <sup>1,3</sup></i>	
ESTIMATING THE LOCAL SPREAD OF LPAI H5N2 BASED ON SEROLOGICAL AND ANTIGEN BASED SERIAL TESTING.....	12
<i>Grewar, J.D.*</i>	
H6 AVIAN INFLUENZA OUTBREAKS IN 2012/2013 IN GAUTENG.....	14
<i>Petty, D.*, Abolnik, C., Rauff, D. &amp; Geertsma, P.</i>	
3 TECH TOOLS FOR STATE AND PRIVATE VETERINARIANS.....	20
<i>Henwood, V.C.<sup>1*</sup> &amp; Taylor, I.<sup>1</sup></i>	
CONTINUING EDUCATION PRESENTATION: AGRICULTURAL TRADE AS A DRIVER OF REGIONAL INTEGRATION IN THE SADC REGION- WHAT ARE THE OBSTACLES? .....	21
<i>Mulumba, M*</i>	
SERO PREVALENCE SURVEY OF <i>NEOSPORA CANINUM</i> IN COWS AND ITS ASSOCIATED RISK FACTORS IN UMUNGUNGUNDLOVU DISTRICT MUNICIPALITY .....	22
<i>Chisi, L.C.<sup>a*</sup>, Mbizeni, S., <sup>b</sup> Perrett, K., <sup>c</sup> Marageni, Y., <sup>a</sup> Naidoo, P., <sup>a</sup> &amp; Zulu, G.<sup>a</sup></i>	
A STUDY OF SOME INFECTIOUS CAUSES OF REPRODUCTIVE DISORDERS IN CATTLE OWNED BY RESOURCE-POOR FARMERS IN GAUTENG PROVINCE, SOUTH AFRICA .....	24
<i>Njiro, S.M., Kidanemariam, A.G., Tsotetsi, A.M., Katsande*, T.C., Mnisi, M., Lubisi, B.A., Potts, A.D., Baloyi, F., Moyo, G., Mpofu, J., Kalake, A. &amp; Williams, R.</i>	
PREVALENCE OF GASTROINTESTINAL HELMINTHS AND ANTHELMINTIC RESISTANCE ON SMALL-SCALE FARMS IN GAUTENG PROVINCE, SOUTH AFRICA .....	25
<i>Tsotetsi, A.M., Njiro, S., Katsande*, T.C., Moyo, G., Baloyi, F. &amp; Mpofu, J.</i>	



PARTICIPATORY METHODS TO CHANGE PERCEIVED RISKS AND INCREASE UPTAKE OF DISEASE PREVENTIVE MEASURES .....	26
<i>Woods, P.S.A*., &amp; Taylor, N.M.</i>	
PERCEPTION OF COMMUNAL FARMERS ON FOOT AND MOUTH DISEASE CONTROL AT THE WILDLIFE/LIVESTOCK INTERFACE OF THE KRUGER NATIONAL PARK .....	27
<i>Lazarus, D.D.<sup>1,5*</sup>, Burroughs, R.<sup>1</sup>, van Schalkwyk, O.L.V.<sup>2</sup>, Mpehle, A.<sup>3</sup>, Thomson, G.R.<sup>4</sup> &amp; Fosgate, G.T.<sup>1</sup></i>	
PARTICIPATORY APPRAISAL OF PESTE DES PETITE RUMINANTS (PPR) OUTBREAKS IN ISEYIN LOCAL GOVERNMENT AREA OF OYO STATE, NIGERIA.....	28
<i>Morakinyo O. A.<sup>1,2</sup>. &amp; Babalobi O.O.<sup>1</sup></i>	
MASS PET STERILISATION INTERVENTIONS IN INDIGENT COMMUNITIES IN THE BOLAND: A LESSON IN VARIATION.....	34
<i>van Helden, L. S.*</i>	
PREVENTION OF COMMUNICABLE DISEASES: THE HUMAN HEALTH PERSPECTIVE .....	36
<i>Blumberg, L.H.*</i>	
ARE FORMALIN-FIXED-PARAFFIN EMBEDDED TISSUES USEFUL FOR THE DIAGNOSIS OF TUBERCULOSIS IN ANIMALS? .....	37
<i>Govender, K.<sup>A,B</sup>, Hlokw, T.<sup>B</sup>, Michel, A.L.<sup>C</sup></i>	
PREVENTIVE ANIMAL HEALTH: HALFWAY INTO THE FIRST DECADE.....	42
<i>Magadla, V.<sup>*</sup>, Baloyi, F.M., &amp; Moyo, G.M.</i>	
CONTINUING EDUCATION PRESENTATION: PPR - ONE FOOT IN THE SADC REGION, WHAT OPTIONS EXIST TO STOP IT IN ITS TRACKS?.....	43
<i>Mulumba, M.<sup>1</sup></i>	
EPIDEMIOLOGY OF RECENT FOOT-AND-MOUTH DISEASE OUTBREAK VIRUSES .....	44
<i>Blignaut, B.<sup>1*</sup>, Mlingo, T.<sup>1</sup>, van Heerden, J.<sup>1</sup>, Lukhwareni, A.<sup>1</sup>, Maree, F.F.<sup>1,2</sup> &amp; Heath, L.<sup>1</sup></i>	
INDIRECT ASSESSMENT OF VACCINE MATCHING FOR SOUTHERN AFRICAN FOOT-AND-MOUTH DISEASE VIRUSES .....	45
<i>Blignaut, B.<sup>1*</sup>, Lukhwareni, A.<sup>1</sup>, Mlingo, T.<sup>1</sup>, Esterhuysen, J.J.<sup>1</sup>, Fosgate, G.T.<sup>2</sup> &amp; Maree, F.F.<sup>1,3</sup></i>	
THE RISK OF DISEASE SPREAD THROUGH WILDLIFE TRANSLOCATIONS .....	46
<i>De Klerk-Lorist, L.*</i>	
HOLISTIC PET CARE PROGRAM – “GIVE YOUR PET A NEW LEASH IN LIFE”: A REVIEW OF PROGRAM IMPLEMENTATION (2008 – 2013) .....	47
<i>Magadla, V.<sup>*</sup>, Moyo, G.M. &amp; Baloyi, F.M.</i>	

# **CONTINUING EDUCATION PRESENTATION: PRIMARY ANIMAL HEALTH CARE CONCEPT**

Moerane,R<sup>1</sup>

## **SUMMARY:**

What is Primary Animal Health Care (PAHC)? “Good animal health and production management practices that are required to maintain health and production undertaken on an on-going daily basis by the livestock handler.”

PAHC, as the first line of defence against disease development and production loss, can only be provided by the person observing and working with the animals on a daily basis and who is able to detect and report the first signs of disease and thus action prevention or treatment in partnership with local veterinary professionals.

A complete PAHC service provided by veterinary professionals to an individual stock owner but mostly to groups of small scale farmers comprises the following:

- Identifying the most important causes of clinical disease or continuous losses affecting a specific group of stock owners within a specific environment with access to a specific set of resources. This is done through regular and structured feedback sessions.
- Veterinary extension services that transfers the identified and relevant knowledge, skills and tools needed at ground level for the implementation of effective PAHC by the stock handler
- Veterinary support services that assist the trained livestock producer on an on-going basis in understanding and responding to the changing animal health challenges faced and
- Access to an identified basic range of stock remedies following on an appropriate knowledge transfer on the basic remedies and their use

Therefore, PAHC Programmes (the ‘Programme’), should provide a structured easy to follow extension methodology and step by step approach to the knowledge, skills and tools needed by the livestock owner to identify, treat and prevent the most common diseases causing death and loss of production.

The presentation will expose members of the profession to models developed to date by Afrivet Business Management and currently being further developed in partnership with the Veterinary Faculty at the University of Pretoria.

# EVALUATION OF ELISA FOR DETECTION OF RABIES SPECIFIC ANTIBODIES IN DOMESTIC DOGS: A PRELIMINARY STUDY

Ngoepe<sup>a\*</sup>, E. & Sabeta, C<sup>a</sup>.

## ABSTRACT

Rabies is preventable through pre-exposure vaccination a process considered successful when the neutralizing antibody titre is  $\geq 0.5$  IU/ml in serum from a vaccinated human and animal. Neutralizing antibodies are therefore a good indicator of successful vaccination to ensure protection against rabies virus infection. The serological assays that are currently recommended and prescribed by the World Health Organisation (WHO) and World Animal Organisation (OIE) to assess the levels of neutralizing antibodies include neutralisation tests mainly the rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralisation test (FAVNT). Both methods are complex and tedious for large scale sero-surveillance studies; in addition they require facilities capable of handling live virus, laboratory with fluorescent microscopy and the expertise to perform these tests and only a few institutions are able to perform these procedures. There are several serological assays that have been developed to measure the level of rabies antibodies in serum samples such as ELISA. The ELISA designed by BioPro was initially developed for use on field samples from foxes to check the efficacy of oral vaccination campaign in Europe. In the current study the sensitivity and specificity were evaluated for testing dog sera from South Africa. It was found that specificity evaluated for 24 unvaccinated individual was 100%, whilst the sensitivity was found to be 83%. The BioPro ELISA could be a reliable method for measuring rabies antibody levels in dog sera from South Africa especially for sero-surveillance studies after vaccination campaigns in the region.

## INTRODUCTION

Rabies is a viral zoonosis caused by negative-stranded RNA viruses belonging to the *Lyssavirus* genus in the *Rhabdoviridae* family, order *Mononegavirales*. The *Lyssavirus* genus comprised of 12 viral species of which five [Classical rabies virus (RABV), Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV) and Shimoni bat lyssavirus (SHIBV)] have been exclusively identified and isolated in Africa (ICTV, 2012). Ikoma lyssavirus (IKOV) has been recently proposed as a new species to the *Lyssavirus* genus isolated from African civet (*Civettictis civetta*) in Tanzania (Marston et al., 2012). Rabies can be successfully prevented through pre-exposure prophylaxis. Pre-exposure vaccination is considered successful when neutralizing antibody titre is  $\geq 0.5$  IU/ml in a serum from vaccinated humans and animals (Wasniewski and Cliquet, 2012). Neutralizing antibodies are a good indicator of successful vaccination to ensure protection against rabies. The serological assays that are currently recommended and prescribed by the World Health Organisation (WHO) and Office International des Epizooties (OIE) to measure the presence of neutralizing antibodies are the rapid fluorescent focus inhibition test (RFFIT) [Smith et al., 1973] and fluorescent antibody virus neutralization test (FAVNT) [Cliquet et al., 1998]. Though they are most widely used methods, both tests are too complex to perform, tedious for large scale sero-surveillance studies, requires facilities to handle live virus and/or expertise to perform these tests and only few laboratories are able to execute these procedures (Sugiyama et al., 1997; Cliquet et al., 2004; Shiota et al., 2009).

For these reasons various techniques have been developed to detect rabies specific antibodies from vaccinated individuals such as enzyme-linked immunosorbent assays (ELISAs) (Elmgren and Wandeler, 1996; Cliquet et al., 2004; Mahumuda et al., 2007). There are several advantages of ELISA techniques over recommended virus neutralization assays namely; rapid, cheaper, easy to perform and results could be available within few hours (Elmgren and Wandeler, 1996; Mahumuda et al., 2007; Shiota et al., 2009). There are few ELISA kits that measure rabies specific antibodies, which are commercially available such as indirect ELISA (BioRad, France) and/or BioPro rabies ELISA antibody kit (O.K. SERVIS Bio Pro S.r.co, Czech Republic). The BioPro rabies ELISA was initially developed and validated for the detection of rabies antibodies in wildlife serum samples and body fluids (foxes) after oral vaccination. Therefore, the aim of the study was to evaluate the specificity and sensitivity of a rabies ELISA kit for dog serum samples from South Africa.

<sup>a</sup> OIE Rabies Reference Laboratory, Agricultural Research Council-Onderstepoort Veterinary Institute, P Bag X05, Onderstepoort, Pretoria, 0110, South Africa.

Contact no: +27 12 5299 439

\*e-mail: NgoepeE@arc.agric.za.

## MATERIALS AND METHODS

### SAMPLES

#### *PANEL OF NAÏVE SERA FROM FIELD ANIMALS*

A panel of 24 sera from unvaccinated domestic dogs were used in the study. The serum samples were heat-inactivated at 56°C for 30 min before testing and stored at -20°C until required. All the serum samples were tested using commercial ELISA kit (BioPro rabies ELISA Ab kit, Prague, Czech Republic) and FAVNT (Cliquet et al., 1998).

#### *PANEL OF SERA FROM VACCINATED ANIMALS*

This panel comprised of 18 dog serum samples selected from the serum archived samples and two serum samples of foetal bovine serum (FBS) origin. The archived sera were received by the laboratory for purposes of international trade or pet export testing for rabies. All sera were heat-inactivated prior to testing using both FAVNT and ELISA kit (BioPro rabies ELISA Ab kit).

### METHOD

#### *FAVN TEST*

The rabies specific neutralizing antibody titres were determined by the FAVNT. The OIE positive reference serum adjusted to 0.5 IU/ml and naïve serum were used as positive and negative controls respectively. Briefly, each serum sample as well as both positive and negative control sera (ANSES, France) were added in four consecutive wells and then serially diluted from 1:3 to 1:432. The challenge rabies virus (CVS-11) containing about 50% tissue culture infective dose (TCID<sub>50</sub>) of 100 in 50 µl was then added to each well. After 60 min of incubation, 50 µl of baby hamster kidney (BHK) cells suspension ( $4 \times 10^5$  cells/ml) was added to each well and the microtitre plates were incubated for 48 hrs at 37°C in a humidified incubator with 5% CO<sub>2</sub>. The microtitre plates acetone-fixed (with 80% acetone) and stained by adding 50 µl of an appropriate dilution of fluorescein isothiocyanate (FITC) anti-rabies monoclonal globulin produced in-house (ARC-OVI) to each well. The titre of each serum sample was expressed in International Units per millilitre (IU/ml) by comparing the test results obtained with those of positive control and 0.5 IU/ml was used as positive threshold.

#### *ELISA TEST*

The Rabies ELISA Ab kit was obtained from BioPro (Prague, Czech Republic). The ELISA kit consists of reagents, negative and calibrated positive (CS1, CS2 and C3) controls. Briefly, each serum samples was diluted to 1:2 according to manufacturer's instruction. About 100 µl of diluted sample was added in duplicate into microtitre plate as well as positive and negative controls. The plates were incubated overnight at 2-8°C with gentle shaking. Six washings were performed after incubation to remove unbound antibodies and other proteins contained in the samples. Then, 100 µl of biotinylated anti-rabies antibodies were added into each well and plates were incubated at 37°C for 30 min with gentle shaking. Unbound biotinylated anti-rabies antibodies were then removed with 4 washings. About 100 µl of streptavidin peroxidase conjugate was added into each well and the plates incubated at 37°C for 30 min with gentle shaking. Four washes were performed after the incubation to remove unbound conjugate. The presence of antigen biotinylated antibody complexes was revealed by adding 100 µl of TMB chromogen solution to each well. The plates were incubated in a dark place for 15-20 min at room temperature with gentle shaking. The enzymatic reaction was stopped by adding a solution of 0.5M H<sub>2</sub>SO<sub>4</sub>. The plates were read at 450 nm and the optical densities (OD) were recorded.

The percentage of blocking (%PB) was calculated for each sample according to manufacturer's specifications:  $(\%PB = [(OD_{nc} - OD_{sample}) / (OD_{nc} - OD_{pc}) \times 100])$ , *OD<sub>nc</sub> is the optical density of negative control, OD<sub>pc</sub> is the optical density of positive control and OD<sub>sample</sub> is the optical density of a sample*). The manufacturer described that a threshold of positivity of 40% for sero-conversions for serum samples from oral vaccination campaigns and a threshold of positivity of  $\geq 70\%$  for international trade or pet export. According to manufacturer's specifications titres of  $\geq 70\%$  are considered as serum samples with antibodies levels equal to or higher than 0.5 IU/ml based on the FAVNT.

### STATISTICAL ANALYSIS

The specificity was assessed on the population of naïve serum samples from unvaccinated dogs having a titre very close to 0 IU/ml. It was expressed as the percentage of true negative results (less than 0.5 IU/ml for the FAVNT and less than 70% for the BioPro ELISA kit). The sensitivity was expressed as the percentage of false

negative results obtained for ELISA kit. A false negative result corresponded to a serum having a positive titre in the FAVNT and a negative titre in the BioPro ELISA kit.

## RESULTS AND DISCUSSION

The comparison between the FAVN test and BioPro ELISA Ab kit test with serum samples is shown in Figures 1 and 2. The cut-off value of 0.5 IU/ml for the FAVNT was used to distinguish positive and negative sera samples. In contrast, BioPro ELISA Ab kit tests cut-off value of 70% as described by the manufacturer, which correspond to the cut-off value of 0.5 IU/ml of FAVNT, was used in the study. The specificity of BioPro ELISA Ab kit test in detecting anti-rabies antibodies for serum samples from unvaccinated dogs was evaluated. The results revealed that all samples from unvaccinated dogs were negative when tested with BioPro ELISA Ab kit (Figure 1). For 24 serum samples tested, BioPro ELISA Ab kit demonstrated a specificity of 100% (Table 1) and the maximum percentage blocking (PB) values obtained was 23%. The specificity of 100% in unvaccinated dogs from field samples could suggest that BioPro ELISA Ab kit is a valuable tool for screening anti-rabies antibodies in vaccinated animals. For the 18 serum samples from vaccinated animals included for analysis 15 were positive and 3 false positive in comparison to FAVN test. Therefore, the sensitivity of BioPro ELISA Ab kit for the vaccinated animals was 83% which is comparable with the previous studies (Wasniewski and Cliquet, 2012). The data presented here clearly demonstrated that BioPro ELISA Ab kit could distinguish the vaccinated animals from unvaccinated animals. However, the three false positive results reported had antibody titre of more than 0.5 IU/ml when measured by FAVNT (Figure 2). Previously, the discrepancy between BioPro ELISA Ab kit and FAVNT was on the samples with a titre of 0.3 IU/ml which is close to 0.5 IU/ml. Therefore further studies are recommended to include cat samples as well to increase the sample size (at least 500 serum samples) which could explain the discrepancy observed in the current study. Therefore, it is recommended that the BioPro ELISA Ab kit could be used as a screening diagnostic assay especially for sero-surveillance studies to determine the protection levels of anti-rabies antibodies in the domestic dog population.

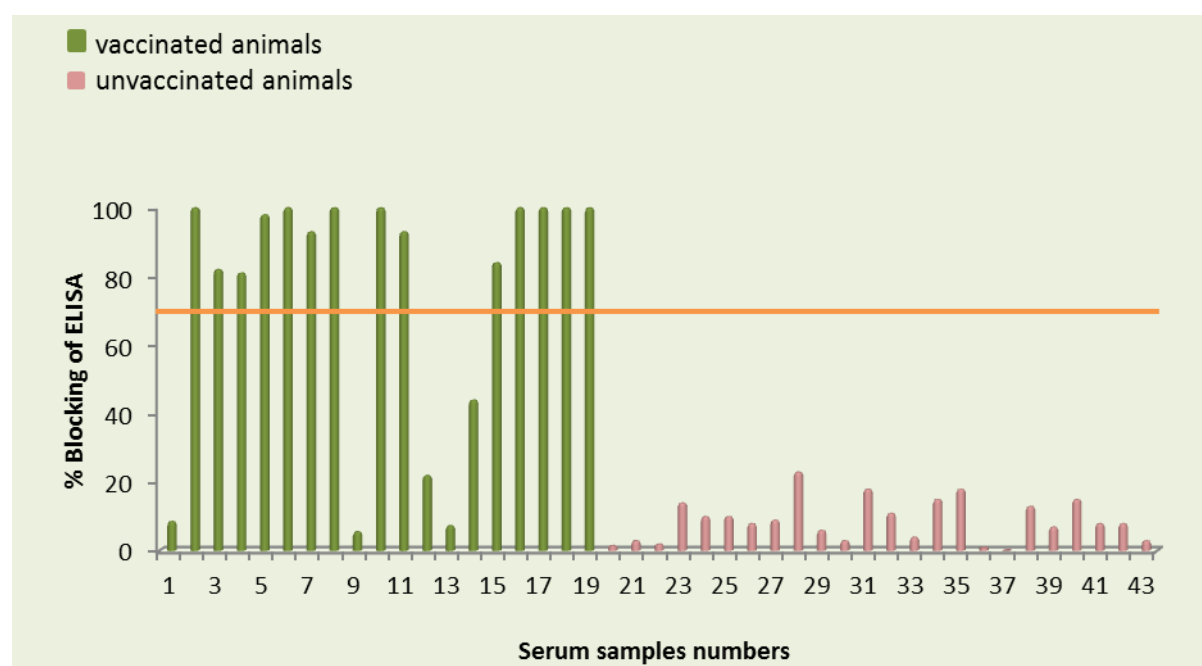


Figure 1. The distribution of percentage of blocking values obtained for the serum samples from vaccinated and unvaccinated animals.



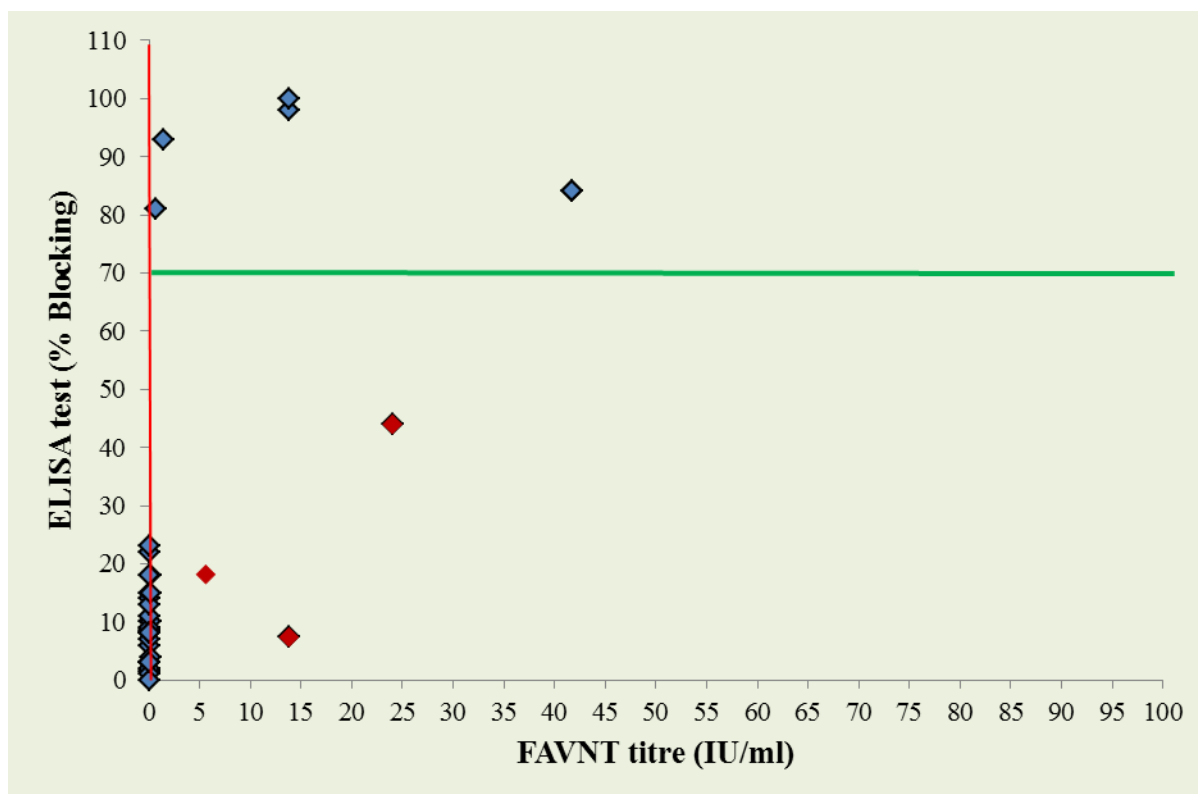


Figure 2. Distribution of antibody titres obtained by BioPro ELISA and FAVNT test. A cut-off value of 0.5 IU/ml for FAVNT and 70% for BioPro ELISA were used to discriminate the protected and unprotected animals included in the study.

Table 1. Cross classification of the ELISA test results by the FAVNT results for the domestic dog serum samples.

FAVNT (0.5 IU/ml cut-off)	ELISA Assay		
	Negative	Positive	Total
Negative	26	0	<b>26</b>
Positive	3	15	<b>18</b>
<b>Total</b>	<b>29</b>	<b>15</b>	<b>44</b>

Sensitivity = 83%

Specificity = 100%

## ACKNOWLEDGEMENTS

This study was funded by European Virus Archive (04/17/C215).

## REFERENCES

- Wasniewski M, Cliquet F 2012 Evaluation of ELISA for detection of rabies antibodies in domestic carnivores. *Journal of Virological Methods* 179: 166-175
- Cliquet F, Aubert M, Sagne L 1998 Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies neutralising antibody. *Journal of Immunological Methods* 212: 79-87.
- Cliquet F, McElhinney LM, Servat A, Boucher J M, Lowings J P, Goddard T, Mansfield KL, Fooks A R 2004 Development of qualitative indirect ELISA for the measurement of rabies virus-specific antibodies from vaccinated dogs and cats. *Journal of Virological Methods*, 117:1-8.
- Elmgren LD, Wandeler AI 1996 Competitive ELISA for the detection of rabies-neutralizing antibodies. In: Meslin FX, Kaplan MM, Kowprowski H (Eds) *Laboratory techniques in Rabies*. World Health Organization, Geneva, pp 200-208.
- Smith JS, Yager PA, Baer GM 1973 A rapid reproducible test for determining rabies neutralizing antibody. *Bulletin of World Health Organization* 48:535-541.
- Sugiyama M, Yoshiki R, Tatsuno Y, Hiraga S, Itoh O, Gamoh K, Minamoto N 1997 A new competitive enzyme-linked immunosorbent assay demonstrates adequate immune levels to rabies virus in compulsory vaccinated Japanese domestic dogs. *Clinical Diagnosis and Laboratory Immunology* 4: 727-730.
- Marston DA, Horton DL, Ngeleja C, Hampson K, McElhinney LM, Banyard AC, Haydon D, Cleaveland S, Rupprecht CE, Bigambo M, Fooks AR, Lembo T 2012 Ikoma lyssavirus, identification of a highly divergent novel Lyssavirus in an African civet (*Civettictis civetta*). *Emerging Infectious Diseases* 18(4): 664-667.
- Shiota S, Mannen K, Matsumoto T, Yamada K, Yasni T, Takayama K, Kobayashi Y, Khawplod P, Gotoh K, Ahmed K, Iha H, Nishizon A 2009 Development and evaluation of a rapid neutralizing antibody test for rabies. *Journal of Virological Methods* 161:58-62.
- International Committee on Taxonomy of Viruses, *ICTV official taxonomy: updates since the 8th report*. *Vertebrate International Committee on Taxonomy of Viruses*, viewed 08 June 2012.

# GENETIC ANALYSIS OF DOMESTIC AND WILDLIFE RABIES VIRUSES FROM THE NORTH-WEST (2012) DEMONSTRATE A COMMON RABIES CYCLE

Sabeta, C<sup>\*</sup>, Phahladira, B., Miyen, J., Mohale, D., Ngoepe, E. & Shumba, W.

## INTRODUCTION

The rabies virus (RABV), a non-segmented negative sense RNA virus and prototype species of the *Lyssavirus* genus (*Rhabdoviridae* family & order *Mononegavirales*), induces a fatal encephalomyelitis in mammals through infectious saliva. This zoonotic and fatal disease is endemic in the Republic of South Africa (RSA) and many other parts of Africa and Asia, where it is responsible for at least 55 000 human deaths annually<sup>3</sup>. In the RSA, The domestic dog maintains RABV throughout the whole country and particularly in the coastal KwaZulu Natal (KZN), Limpopo (LP), Mpumalanga (MP) and Eastern Cape (EC) provinces, whereas the black-backed jackal (*C. mesomelas*) and the bat-eared fox (*Otocyon megalotis*) are pivotal in the maintenance and transmission of the disease in the northern and western parts of the country<sup>7</sup>. In addition to canid rabies biotype (maintained by dogs and wildlife hosts, the bat-eared fox and black-backed jackal species), the mongoose rabies biotype (or herpestid variant) maintained by the yellow mongoose *Cynictis penicillata* (*Herpestidae* family) is prevalent on the highveld plateau of South Africa<sup>6, 9</sup>. Spill-over of infection occurs between domestic (canid) and wildlife (mongoose) carnivores in South Africa, with spill over from wildlife (mongoose to canid) being more common than the reverse<sup>2, 5</sup>. Given that 99% of the human rabies cases accounted for globally are dog-mediated, elimination of the diseases in the endemic regions such as in South Africa is further complicated by the presence of wildlife rabies cycles.

## MATERIALS AND METHODS

A retrospective analysis of rabies submissions from the North-West province was undertaken. The samples analysed in this study were recovered from the specimens submitted for routine rabies diagnosis at Onderstepoort Veterinary Institute (Pretoria) and initially shown to contain lyssavirus antigen by the direct immunofluorescent antibody test<sup>1</sup> (dFAT). Subsequently, antigenic characterisation of the positive samples was performed with a panel of 15 anti-nucleocapsid monoclonal antibodies (N-Mabs) (CFIA, Ontario, Canada). For genetic analysis, total viral extractions were done using Trizol according to the supplier's guidelines (Sigma, U.S.A) and the RNA subjected to reverse-transcription by targeting a partial region of the nucleoprotein gene (N). The target region was then amplified using the LysOO1 (+) and 550B (-) primer pair using cycling conditions described previously<sup>4</sup>. High quality amplicons were purified using spin columns and sequenced [ABI Big Dye Terminator V3.1 sequencing kit, Applied Biosystems) using both the forward and reverse primer set as used in the reverse-transcription PCR steps. The forward and reverse nucleotide sequences were edited and consensus sequences assembled and phylogenetic analysis undertaken using algorithms in MEGA<sup>8</sup>.

## RESULTS AND DISCUSSION

North-West Province is one of the regions in the RSA with low reported and confirmed rabies cases, both in domestic and wildlife species. An analysis of rabies cases over a 5-year period [2008-2012] showed that slightly more positive samples from wildlife submissions [56%] compared to domestic [44%] host carnivores were received at the OIE Rabies Reference Laboratory. When the nucleotide sequences of the partial region gene was compared to those recovered previously from other regions of the country, a single cluster, statistically supported by a 95% bootstrap support value and consisting of 5 RABVs, one each from a canine from (LP) and the NW, two from a jackal species (*C. mesomelas*) from the NW and a single isolation from a hyaena *Crocuta crocuta* (also from the NW) was evident. This observation probably demonstrates a very close link and cross-species transmission of the RABV infection between domestic and wildlife hosts in this part of the country. These data further underscore the complexity of rabies epidemiological cycles in South Africa and the challenges posed for the elimination of the disease from both domestic and wildlife reservoirs and vector species. Clearly, an increasing prevalence of wildlife rabies with various wildlife species emerging as the maintenance host species complicates and prohibits the successful elimination of the disease from many regions in the world and certainly in most of Africa<sup>10</sup>. Despite the immunization of domestic dogs, it appears that rabies epizootics will be sustained in these areas by jackals and any other wildlife species that will subsequently act as source of infection for domestic dogs thus posing a serious public health threat.

<sup>\*</sup>OIE Rabies Reference Laboratory, Agricultural Research Council-Onderstepoort Veterinary Institute, P Bag X05, Onderstepoort, Pretoria, 0110, South Africa.

Contact no: +27 12 5299 439

e-mail: SabetaC@arc.agric.za.

## REFERENCES

1. Dean D J, Abelseth M K, Atanasiu P 1996 'The fluorescent antibody test', in FX Meslin, Kaplan M M, Koprowski H (eds), Laboratory techniques in rabies, World Health Organization, Geneva.
2. King A A, Meredith CD, Thomson GR 1993 Canid and viverrid viruses in South Africa. *Onderstepoort J Vet Res* 60: 295-299.
3. Knobel D L, Cleaveland S, Coleman P G, Fèvre E M, Meltzer MI, Miranda M E G, Shaw A, Zinsstag J, Meslin F X 2005 Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 83:360-368
4. Markotter W, Kuzmin I, Rupprecht C E, Randles J, Sabeta C T, Wandeler A I, Nel L H. Emerg Infect Dis. 2006; 12(12):1913-8.
5. Nel L H, Jacobs J A, Jaftha J B, Meredith C D 1997 Natural spillover of a distinctly canidae-associated biotype of rabies virus into an expanded wildlife host range in southern Africa. *Virus Genes* 15:79-82
6. Skinner J D, Smithers R H N 1990 The mammals of the southern African subregion. University of Pretoria, Pretoria.
7. Swanepoel R, Barnard B J H, Meredith C D, Bishop G C, Brückner G K, Foggin C M, Hübschle O J 1993 Rabies in Southern Africa. *Onderstepoort J Vet Res* 60: 325-346
8. Tamura K, Dudley J, Nei M, Kumar S 2007 MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software Version 4.0. *Mol Biol Evol* 24: 1596-1599
9. Taylor P J 1993 A systematic and population genetic approach to the rabies problem in the yellow mongoose (*Cynictis Penicillata*). *Onderstepoort J Vet Res* 60:379-387
10. Zulu G C, Sabeta C T, Nel L H 2009 Molecular epidemiology of rabies: focus on domestic dogs (*Canis familiaris*) and black-backed jackals (*Canis mesomelas*) from northern South Africa. *Virus Research* 140 (1-2): 71-78

*ARTICLE PUBLISHED*

SABETA, C.T., WEYER, J., GEERTSMA, P., MOHALE, D., MIYEN, J., BLUMBERG, L.H., LEMAN, P.A., PHAHLADIRA, B., SHUMBA, W., WALTERS, J. & PAWESKA, J.T., 2013, 'EMERGENCE OF RABIES IN THE GAUTENG PROVINCE, SOUTH AFRICA: 2010-2011', *J.S.AFR.VET.ASSOC.* 84(1), E1-E5.

## **EMERGENCE OF RABIES IN THE GAUTENG PROVINCE, SOUTH AFRICA: 2010-2011**

Sabeta, C.T., Weyer, J., Geertsma, P., Mohale, D., Miyen, J., Blumberg, L.H., Leman, P.A., Phahladira, B., Shumba, W., Walters, J. & Paweska, J.T

### **ABSTRACT FROM PUBLISHED ARTICLE**

Canine rabies is enzootic throughout Sub-Saharan Africa, including the Republic of South Africa. Historically, in South Africa the coastal provinces of KwaZulu-Natal and Eastern Cape were most affected. Alarming, outbreaks of canine rabies have been increasingly reported in the past decade from sites where it has previously been under control. From January 2010 to December 2011, 53 animal rabies cases were confirmed; these were mostly in domestic dogs from southern Johannesburg, which was previously considered to be rabies free. In addition, one case was confirmed in a 26-month old girl who had been scratched by a pet puppy during this period. The introduction of rabies into Gauteng Province was investigated through genetic analysis of rabies positive samples confirmed during the outbreak period. In addition, the nucleotide sequences of incidental cases reported in the province for the past ten years were also included in the analysis. It was found that the recent canine rabies outbreak in the Gauteng Province came from the introduction of the rabies virus from KwaZulu-Natal, with subsequent local spread in the susceptible domestic dog population of southern Johannesburg. The vulnerability of the province was also highlighted through multiple, dead-end introductions in the past ten years. This is the first report of a rabies outbreak in the greater Johannesburg area with evidence of local transmission in the domestic dog population

# **RABIES STATUS IN KWAZULU NATAL**

Le Roux, K<sup>1</sup>.

## **ABSTRACT**

Selected out of ten competing countries, KwaZulu-Natal veterinary services has received financial and intellectual input from the Bill and Melinda Gates foundation and other international organizations such as the WHO, into a rabies elimination showcase project. The objectives of the project are to demonstrate the effective elimination of human rabies, through the control or elimination of the disease in the host canine species. The project then aims to produce a blueprint of how this program can be expanded in Africa and other developing countries around the world.

This presentation focuses on the research that has emanated from the project, through the evaluation of all aspects pertaining to the disease and its control. From Molecular epidemiology, through dog ecology to detailed evaluation of socio-political aspects that influence control measures, rabies has been brought under control in the province and elimination of canine rabies is now a reality.

<sup>1</sup> Rabies Project Manager – KZN Veterinary Services, Pietermaritzburg, South Africa

## **CONTINUING EDUCATION PRESENTATION: IMPACT OF TRAINING USING A STRUCTURED PRIMARY ANIMAL HEALTH CARE MODEL ON THE SKILLS OF RURAL SMALL SCALE FARMERS**

Moerane, R.,<sup>\*1</sup> Terblanche, S.E.,<sup>2</sup> Irons, P.C.<sup>1,3</sup>

### **SUMMARY**

Primary animal health care (PAHC) programmes aimed at the provision of basic animal health services at local level follow a variety of approaches or programmes among various veterinary and para-veterinary professionals and there is no common approach or framework in South Africa. A training model has been developed by Afrivet Training Services (ATS). The aim of this study was to evaluate the impact of training using the ATS PAHC model, on the skills of small scale rural farmers.

Ninety farmers were selected randomly from lists of all farmers in the Makapanstad, Ratjiepane and Mnisi areas and ultimately 78 farmers participated. The selected farmers were assessed for knowledge and skills with background information collected prior to training sessions. The statistical significance was assessed at  $P < 0,05$ .

The results of the study indicate that complete data was available for 77 participants, of which the majority were adult males with a mean age of 49 yrs. There was a significant involvement of youth at 31% and females at 32%. The unemployment rate amongst participants was 91%. Farmers continue to perceive clinical services as being unaffordable, with most (52%) participants preferring to treat animals themselves and 27% approaching the nearest farmer and only 14% firstly seeking assistance from a veterinarian. Lack of knowledge, poor access to products and poor communication between the farmers and the veterinary officials are major obstacles in maintaining healthy animal population.

The training had an immediate impact as the farmers gained knowledge and skills to observe, examine, treat and implement preventative measures on their own and there was a better understanding of their role in the provision of animal health care. There were significant differences between the genders in the acquisition of specific skills during the training, with females performing better in data recording while males were more skilled at determining temperature using a thermometer.

Our conclusion from the study is that the training model used had a positive impact on the skills of the trained farmers. Training also assists small scale farmers to handle problems with guidance from a veterinary professional. A modified, standardised method to evaluate knowledge before and after training is proposed for future studies. The farmers in this study perceive clinical service as an expensive venture and very few of the participants utilise the services of a veterinarian. Through such a training programme, participants start valuing the role and impact of a veterinary professional in the area. Therefore, training on basic animal disease identification and treatment is proposed before expanding on clinical services in an area.

<sup>1</sup> Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria;

<sup>2</sup> Department of Agriculture, Extension and Rural Development,

<sup>3</sup> Institute of Food, Nutrition and Wellbeing, University of Pretoria

\*email: [rebone.moerane@up.ac.za](mailto:rebone.moerane@up.ac.za)

*THE TEXT BELOW IS IN AN EXTENDED ABSTRACT FORMAT. A DETAILED SUMMARY HAS BEEN PUBLISHED IN THE WESTERN CAPE DEPARTMENT OF AGRICULTURE EPIDEMIOLOGY REPORT.*

*GREWAR, J.D. 2013 ESTIMATING THE LOCAL SPREAD OF LPAI H5N2 BASED ON SEROLOGICAL AND ANTIGEN BASED TESTING WESTERN CAPE GOVERNMENT: AGRICULTURE: VETERINARY SERVICES EPIDEMIOLOGY REPORT JAN 2013 VOL 5 ISS 1 PPI-7*

## **ESTIMATING THE LOCAL SPREAD OF LPAI H5N2 BASED ON SEROLOGICAL AND ANTIGEN BASED SERIAL TESTING**

Grewar, J.D\*.

### **INTRODUCTION**

During the course of the highly pathogenic H5N2 avian influenza (HPAI) outbreak the Western Cape experienced during 2011 there were other avian influenza viruses detected during the course of routine and outbreak response surveillance. Included in these events were outbreaks of low pathogenic H5N2 avian influenza (LPAI) on ostrich farms in the 3 major ostrich farming regions of the Province, namely the Klein Karoo, the Mossel Bay region and then the Southern Cape with a focus in the Albertinia and Heidelberg regions.

The broad case definition for LPAI H5N2 farms involved both antigen detection (PCR) and serological testing (ELISA and HI), but epidemiological links between suspect farms and confirmed LPAI H5N2 farms was also included in the definition. This was necessary because of farms which tested serologically positive to H5 AI antibodies with no further AI subtyping based on PCR or sequencing - thus these farms needed to be excluded as potential HPAI farms as the HPAI outbreak was also as a result of an H5N2 virus.

In this article the focus was on the potential direction and intensity of spread and infection of LPAI H5N2 avian influenza between separate groups of ostriches on an ostrich farm in the Oudtshoorn district (hereafter referred to as Farm #439) which tested LPAI H5N2 positive early in 2012. This spread and intensity is estimated using serological and PCR prevalences per group evaluated over time.

### **MATERIALS AND METHODS**

#### **FARM #439**

Farm #439 was a raising to slaughter ostrich unit in the De Rust area in the Klein Karoo. It was part of a larger production unit where a breeder farm produces eggs which are hatched and day old chicks are used to supply two raising farms, #439 being one of these. There was a specific flow of animals on the farm with young chicks entering the system on the so called 'South Farm' past of #439 and eventually growing to slaughter birds which were fed and sent to slaughter from the 'North Farm'. Age group associated camps of varying sizes were situated throughout both parts of the farm.

#### **SELECTION OF SAMPLING GROUPS**

The farm was first detected AI positive after sampling on the 3 February 2012 was performed. This sampling was initiated during a survey (8<sup>th</sup> round survey) performed in the Avian Influenza Control Area (AICA) in the Klein Karoo valley. This control area was instituted as a direct result of the HPAI H5N2 outbreak in that region. Both serum and tracheal swabs were taken from each farm during that survey and in this case 30 samples were taken from 2 age groups on the farm. Based on the initial results a full farm level sampling was performed to estimate the extent of the virus. Results from this sampling event indicated a definite active AI circulation on the farm, particularly in the 'North Farm'. One camp was then chosen per age and space separated epidemiological group on the farm at this point to further estimate the spread patterns of AI on the farm. The number of separate groups evaluated totalled five: one slaughter age group, two medium aged chicks groups and two young chick groups. All groups were from the 'North Farm' bar one – the young chick group.

The follow up sampling protocol consisted of sampling the selected groups on the farm on a two weekly basis. A decision was however made to have the farm slaughtered out as a consequence of the LPAI virus and so a final sampling was also then performed just prior to slaughter out of each group.

\*Western Cape Department of Agriculture: Veterinary Services

Private Bag X1, Elsenburg, 7607

Tel: 021 808 5056

email: johng@elsenburg.com



## ESTIMATING SPREAD OF VIRUS ON THE FARM

Group specific prevalence curves with consolidated time axes of weekly intervals were used to evaluate the spread patterns of the infection. These curves have not been included here but will be discussed in the presentation.

## RESULTS AND CONCLUSIONS

The origin of the virus onto Farm #439 could not be confirmed. The initial introduction onto the farm was either into a medium aged chick group or a slaughter age group which were in close proximity to each other. If the introduction was not into both groups concurrently then the initial spread between these two groups was relatively quick.

The secondary spread occurred into the medium aged and young chick groups on the 'North Farm'. The secondary spread can be directly explained by the close proximity of these camps to the slaughter age camps. Feeding of the birds was performed using a tractor which moved between the medium aged birds and the slaughter age camps in the 'North Farm'. They would then feed the young chicks on the North farm. Fomite spread on workers and/or vehicles would explain the simultaneous spread to the susceptible groups remaining in the North farm after the initial introduction of virus.

The tertiary spread of virus is estimated based on the sudden PCR positive prevalence in the 'South Farm' young chicks observed in samples taken just prior to their slaughter. The distance between the 'North farm' camps and the 'South farm' positive group camp was between 2 and 3 km so local spread on farm by wild water birds like Egyptian geese or by Passerines, along with fomite transmission by workers or vehicles on the farm is possible.

Based on the evaluation of data the following conclusions can be made

1. LPAI H5N2 of the 2012 Western Cape outbreak, while not clinically apparent in ostriches, caused significant seroconversion in susceptible groups of ostriches of at least the production ages from chicks to slaughter age
2. While the serological prevalences within affected groups of birds reached plateau phases in the region of 2 weeks but could take at least 34 days to occur, although the age of the affected group may play a role here
3. The rising serological group prevalences are concurrent with decreasing PCR group prevalences
4. The virus showed significant PCR level prevalences if tests are performed during the PCR positive phase of infection
5. The period of detection using serology is likely to be longer than that of PCR detection alone
6. The farm level detection of AI is likely to be successful using serology alone unless the PCR positive phase alone is present early on in the farm infection
7. The introduction of AI onto this farm was unknown but likely to be from a wild water bird source
8. The local spread on the farm however was more likely to have been fomite spread via people working with the birds or via feed transport vehicles

## ACKNOWLEDGMENTS

This study would not have been possible without the hard work involved with sampling and testing of samples. In this regard I acknowledge the animal health technicians involved during the investigation - Mr Flip Kemp (who took the initial samples which resulted in the follow up), Mr Alwyn Krause and Mr Attie Erasmus. Mr Carel Lotz who assisted with sample handling and processing and Mr Dawid Visser who managed the logistics of sampling and also managed the negotiations with the farmer involved. Claudia Falch from Deltamune was intrinsically involved in all aspects of the testing of both swab and serum samples as well as offering to test all serum samples on the HI assay to prevent false negative ELISA's from confusing HI prevalences.

I acknowledge the assistance of Prof Ian Brown and Dr Stefano Marangon who assisted in the initial investigation and gave guidance in the follow up protocol

I acknowledge the input of officials from the Western Cape Department of Agriculture and the Department of Agriculture, Forestry and Fisheries.

I also acknowledge the assistance given from the farmer involved in his willingness to assist us with data collection and explanation of aspects of his farming.

# H6 AVIAN INFLUENZA OUTBREAKS IN 2012/2013 IN GAUTENG

Petty, D.\*, Abolnik, C., Rauff, D. & Geertsma, P.

## ABSTRACT

We report on H6 Avian Influenza (AI) outbreaks on Gauteng poultry farms in 2012, 2013. In most instances the H6 was detected on routine serosurveillance and was not associated with significant symptoms. The virus was recovered in only 3 instances and was sequenced. It appears that there has been significant drift of the H6 virus over time, as well as an altered pathogenicity. We conclude that it is important to monitor genetic drift of H6 in AI outbreaks and reduce the opportunity for H6 virus to reassort with viruses from wild birds.

## INTRODUCTION

Avian influenza is of great concern worldwide. There are 17 known influenza A virus hemagglutinin (HA) subtypes (H1 to -16) and nine neuraminidase (NA) subtypes (N1 to -9), most of which have been isolated from aquatic birds and one new serotype in bats, H17N10, was recently described (Li et al., 2012). AI virus infections show a continuous spectrum of pathogenicity in gallinaceous poultry, from no pathogenicity to high pathogenicity (Australian Veterinary Emergency Plan, 2011). Clinical signs in chickens and turkeys range from inapparent to mild or severe respiratory disease. Mortality from LPAI ranges from 3% in caged layers to 15% in broilers; high mortalities up to 90% have been recorded in young turkey poults.

LPAI infections of chickens and turkeys with H5 and H7 subtype that have been allowed to continue without adequate control or eradication procedures have ultimately turned into virulent HPAI infections (Pennsylvania, United States, 1982–83; Mexico, 1994; Italy, 1999–2000). The change in virulence of the virus is associated with the acquisition of additional basic amino acids at the cleavage site of the haemagglutinin protein (Australian Veterinary Emergency Plan, 2011). Although H5 and H7 are associated with human cases and are seen to have pandemic potential, it is not possible to predict what the next pandemic will be and what type of influenza it will involve.

The first H6 influenza virus was isolated from a turkey in 1965, and since then H6 viruses have been isolated with increasing frequency from wild and domestic aquatic and terrestrial avian species throughout the world (Gillim-Ross, et al., 2008). H6 viruses continue to circulate worldwide in areas including North America (Kinde, et al., 2003) and South Africa (Abolnik, et al., 2007) as well as in Asian countries including southern China and Taiwan (Kim, et al., 2010). There are 2 lineages of H6 namely the Eurasian and American lineage and they tend to cluster with respect to geographical location. H6N2 influenza viruses were isolated from California chickens in 2000 and 2001. These H6N2 viruses were non-pathogenic in experimentally infected chickens and could be divided into three genotypes (Webby, et al., 2002). The presence of multiple H6N2 genotypes suggests that independent transmission and/or reassortment events may have taken place between aquatic bird and chicken influenza viruses. Mortality in infected flocks ranged from 0.25% to 3%, and egg production dropped between 7% and 40% (Kinde, et al., 2003).

All of the H6 isolates that have been examined have been of low pathogenicity. The virus normally causes asymptomatic infections in waterfowl. (Advisory Committee on Dangerous Pathogens, 2008). In a study involving Europe and America, H6 was the most abundant isolate in wild birds. In China H6 is regarded as endemic and is the most abundant subtype and is present all the year round. The prevalence of H6 viruses in aquatic and terrestrial birds increases the potential for transmission to humans (Gillim-Ross, et al., 2008). No natural human infections have been reported with the H6 subtype, however An H6 influenza virus was identified as a potential progenitor of the H5N1 viruses that emerged in Hong Kong in 1997 (Gillim-Ross, et al., 2008).

H6 strains differ in their virulence as well as their immunogenicity. In contrast to H5 and H7 lethality in the H6 strain is not associated with extra-pulmonary spread. H6 strains caused significant mortality in mice and ferrets and induced mild respiratory signs in human volunteers that were inoculated with it. Shedding of virus after infection depends on the strain but may vary from 5 days for a Korean H6N5 isolate (Nam, et al., 2011) and given as 7-10 days in a DEFRA document (Advisory Committee on Dangerous Pathogens, 2008)

Immunity following infection with a virus of the same H subtype persists for varying lengths of time. The United States government control programs during the 1983–84 outbreak in Pennsylvania demonstrated that some flocks that had seroconverted but were not showing clinical signs of AI were generally seronegative six weeks after they were assumed to have been infected. However, other infected flocks that recovered were seropositive for up to a year later, although virus could not be isolated. (Australian Veterinary Emergency Plan, 2011)

Antigenic and genetic analyses of the H6 influenza viruses isolated from domestic poultry in south-eastern China from 2001 through 2003 provide convincing evidence that the H6 influenza viruses are reassorting with

\*Contact Author- Gauteng Department of Agriculture and Rural Development

9 Diagonal Street, Johannesburg, 2000

Tel: 0833910686

[Dervyn.Petty@gauteng.gov.za](mailto:Dervyn.Petty@gauteng.gov.za)

H9N2, H5N1, and other viruses in the region (Ozaki, et al., 2011). In a Korean study (Kim, et al., 2010), H6N2 viruses isolated from poultry were found to form 3 genotypes as the result of a triple assortment from other LPAI viruses (H9N2 and H3N2).

Jackwood *et al* infected ducks and chickens with a number of isolates of H6 and detected all H6 isolates in the oral-pharyngeal swabs from chickens at 2 and 4 days PI, but only three of the five viruses were detected at 7 days PI. Only two viruses were detected in the cloacal swabs from the chickens. Both the chickens and the ducks developed antibodies, as evidenced by HI and ELISA titres. In other studies it was concluded that LPAI of wild bird origin including H6 can infect chickens but induced mild lesions and poor seroconversion (Morales, et al., 2009).

The World Health Organization has listed H6 as one the avian influenza subtypes with pandemic potential. The list, in priority order, is H5, H7, H9, H2, H6, and H4 (Advisory Committee on Dangerous Pathogens, 2008).

The first detected outbreak of H6 in South Africa arose in 2003 (Abolnik, et al., 2007). Both sublineages of this H6N2 that were circulating in the Camperdown area were shown to have arisen from the reassortment of two ostrich viruses (H9N2 and H6N9) (Abolnik, et al., 2007). Since then there have been numerous outbreaks of H6 in Kwazulu Natal.

## MATERIALS AND METHODS

All detected outbreaks of H6 Avian Influenza (AI) were analyzed and recorded. H6 AI was detected in blood samples using the national protocol which dictates that samples that were tested for AI are tested using and ELISA method for the detection of AI, followed by Haemagglutination Inhibition to confirm the Hemagglutinin type of AI. In all cases, samples were taken for polymerase chain reaction (PCR) and if PCR detected virus, virus isolation was undertaken and the full genome of the virus was sequenced by random amplification deep sequencing as described previously (Abolnik *et al.*, 2012).

Upon detection, all farms were quarantined until no live virus could be detected by PCR and the risk of transmission was considered to be low. In some cases vaccination was undertaken under the control of the Department of Agriculture Forestry and Fisheries with the placement of sentinels which were monitored monthly.

## RESULTS

11 farms were quarantined for H6 between September 2012 and the beginning of August 2013 in Gauteng. The distribution of the outbreaks per month is shown in figure 1.

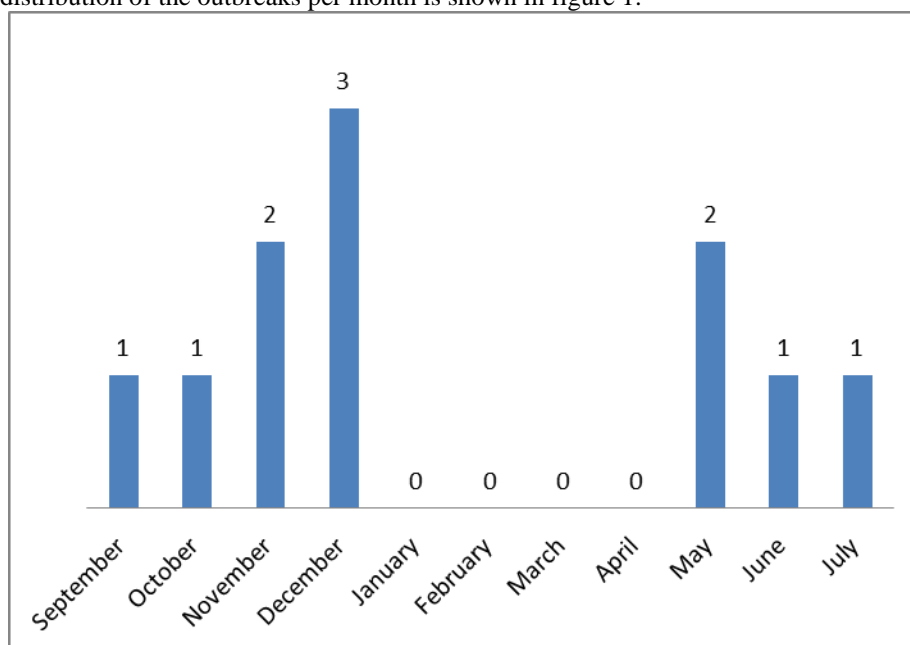


Figure 1: Number of Farms quarantined in Gauteng for H6 Avian Influenza in 2012/2013

Of these farms the majority were layer farms or breeder farms as shown in figure 2.

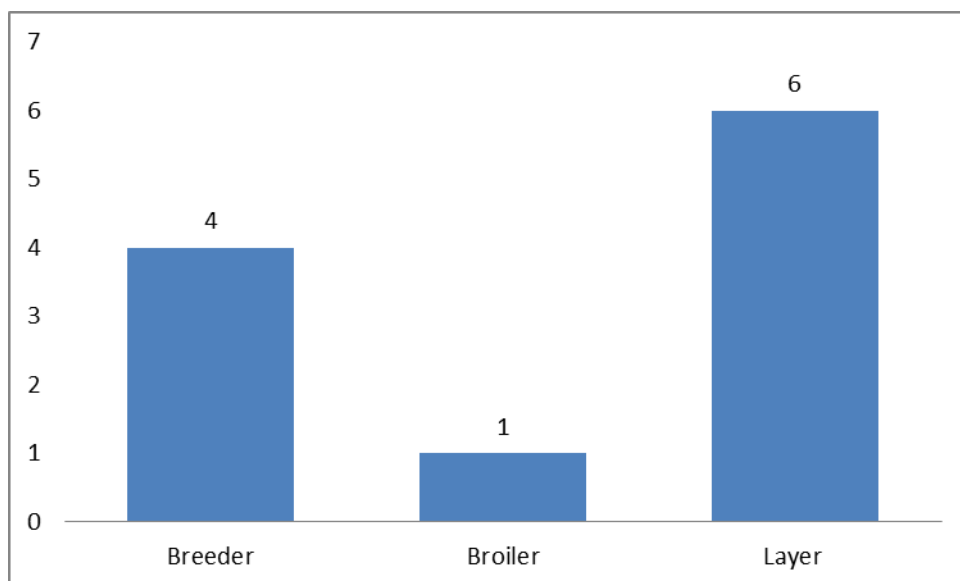


Figure 2: Type of poultry farms that were quarantined with H6

The spatial distribution of the quarantined H6 farms is shown in the map of Gauteng below as filled circles.

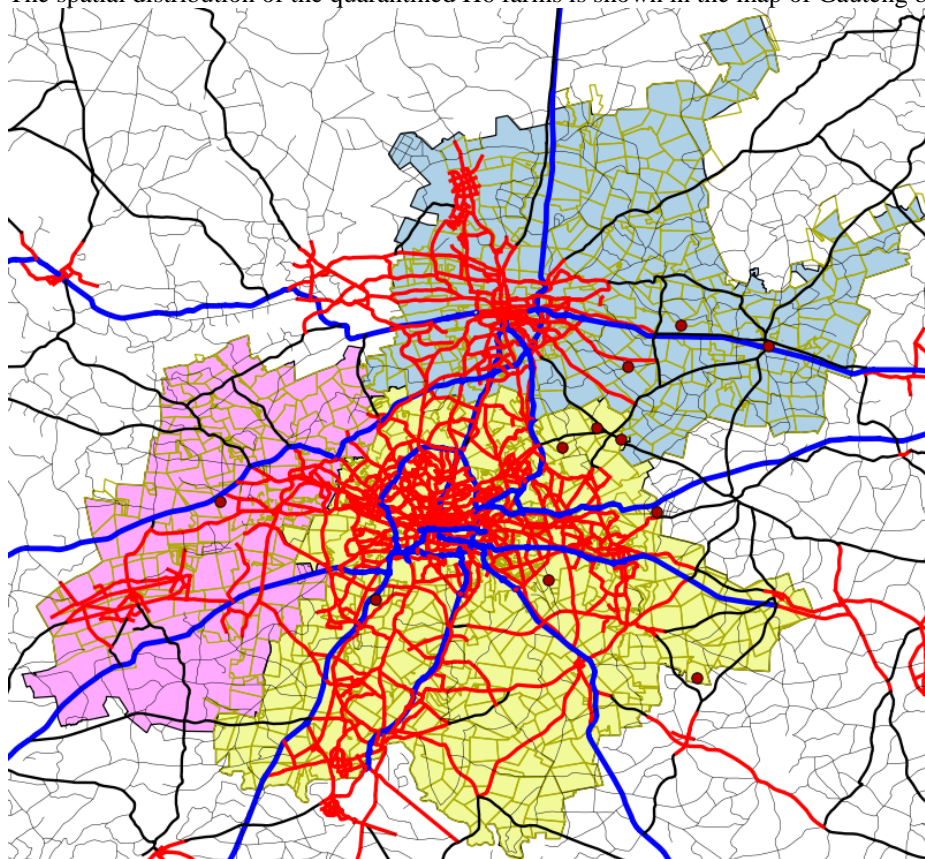


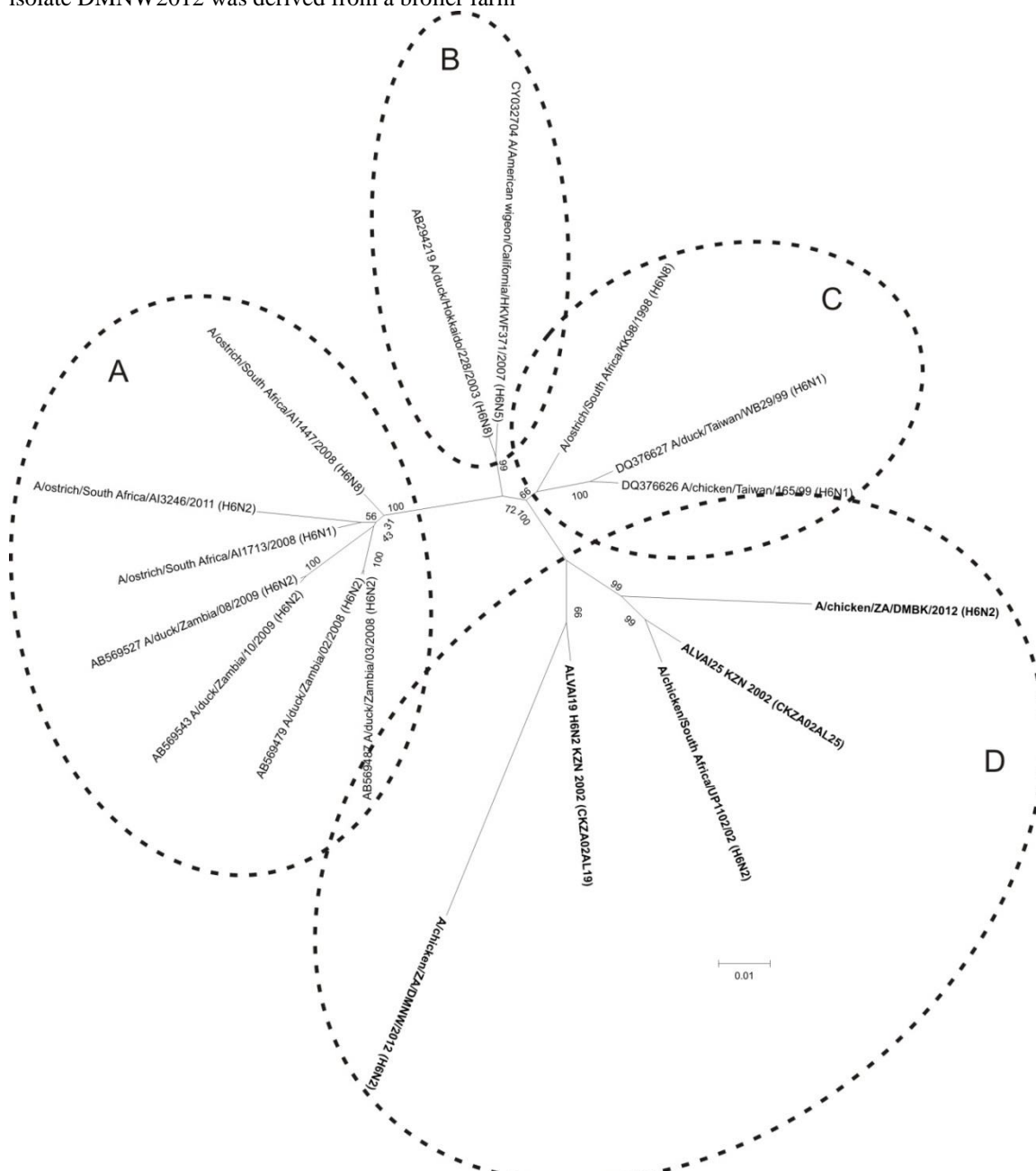
Figure 3 Distribution of H6 outbreaks in GP

With the exception of the broiler farm, there are numerous relationships between the various farms. These include a link with Natal operations where H6 is known to be rife, belonging to the same company, being within 15km of another outbreak. There was a cluster of outbreaks in the Bapsfontein area where poultry workers from all the companies lived in the same village.

A feature of the current H6 outbreaks is the relative lack of symptoms in the birds. Only 3 out of the 11 outbreaks were associated with drops in production or decreased feed consumption or respiratory symptoms. In all cases the clinical picture was relatively mild. In the majority of cases H6 was detected on routine serology

and at that stage, all attempts to isolate the virus proved futile. In all 11 quarantines, only in 3 cases were the viruses isolated and in 2 instances these were sent for sequencing.

The phylogenetic tree is presented below in figure 4. Isolate DMBK2012 is derived from a breeder farm while isolate DMNW2012 was derived from a broiler farm



**Figure 4: Phylogenetic tree of H6 hemagglutinin genes. Clade A= contemporary southern African H6 isolates from wild ducks and ostriches; Clade B=other international lineages; Clade C=ancestral H6 viruses to South African chicken strains; Clade D=South African chicken H6N2 viruses from 2002 and 2012.**

Vaccination with a killed H6N2 vaccine (<sup>TM</sup>Avivac) was undertaken for birds entering 2 of the 11 farms. As required by the DAFF policy, when this is done, sentinels are placed and monitored monthly.

## DISCUSSION

Prior to September 2012, the last farms to be quarantined were the 2 farms in the Midrand area in late 2008 and 2009 respectively. In both cases the birds were moderately to severely affected with 30 – 40% production drops and white eggs. In 2012/13 there were 11 farms quarantined as the result of H6. Given that the disease was often only detected on routine serology, this is likely to be a significant under detection.

It is likely that the increased frequency of detection of H6 is at least in part due to the application of the testing protocol which requires that samples for avian influenza are tested with a general ELISA followed by a specific HI test to identify the virus as the which H and N antigens are present.

Another feature of the current outbreaks is that in many cases the disease was very mild or even in apparent and was only detected on routine serology. Virus isolation was often negative by then. Nevertheless, the virus was isolated in 2 instances. In the first case the breeder farm had drops in production and increased mortalities during which time the virus spread rapidly from site to site on the farm. In the second instance, the virus was isolated in routine samples taken from the abattoir. Examination of the haemagglutinin genes and the neuraminidase genes in both of these isolates reveal significant drift but no reassortment with other H6 isolates from wild birds or ostriches. This is perhaps not unexpected given the very different clinical pictures that presented in these two cases.

All the farms were placed under quarantine until it could be shown that there was no virus present. In many cases, since no virus was ever detected, it is likely that there was no control during the period when the birds were shedding and thus quarantine as a tool to contain H6 is not effective. Thus routine biosecurity measures have to be effective enough to prevent any outbreak and contain an outbreak of H6 should it occur.

Avian Influenza is defined by the EU as an infection of poultry with H5 or H7 or an infection with an IVPI of 1.2. This definition excludes H6 and thus H6 control is left to the individual states. Within Europe, culling is not always undertaken in an economically viable flock if a low pathogenicity avian influenza virus is identified. The disease may be allowed to run its course within a flock. This is because in most instances the birds do recover and egg production can return to normal (Advisory Committee on Dangerous Pathogens, 2008). In order to minimise the risk of exposure of humans, it was recommended in the UK that infected turkeys be culled. However it was acceptable if only older turkeys were slaughtered and the younger turkeys were allowed to grow out (followed by later slaughter) provided that: - full PPE be maintained for all workers having occupational exposure to potentially infected turkeys, - health surveillance be maintained on all those already exposed, and those subsequently exposed, including the reporting of symptomatic illness and that this should continue for seven day after last exposure to the birds, their waste or bedding. - the collection of paired blood specimens (>14 days apart) for serology is undertaken (Advisory Committee on Dangerous Pathogens, 2008).

In the USA H6 is not a controlled disease and is handled by the industry itself rather than the state. The following recommendations are however made. Initially depopulation of poultry farms with H6 was undertaken but this was not seen to be effective (Swayne, 2003). After that quarantine was used. Table eggs were allowed to be marketed as long as dedicated equipment was used for collection. This was found to be effective. Quarantine was often as the result of serological conversion and was lifted after negative virus isolation.

Although there is a lot of virus in the reproductive tract in layers in H6, virus isolation was unable to confirm the presence of H6 in the eggs, either in the shells or their contents. LPAI virus, hatching eggs were not traced back and destroyed, but strict biosecurity measures and disinfection procedures were defined and enforced both on the farm and at the hatchery. AIV contained in the manure will be inactivated within 1 to 3 weeks depending on temperature; increasing the house temperature will speed up the inactivation process. Experimental studies indicated AIV was inactivated within 6 days in the field in chicken manure at an ambient temperature 15 degrees C. During the AI outbreak two broiler breeder flocks affected with AIV were all depopulated 14 days after the outbreak; the two houses were closed with manure inside. AIV was not detected after 3 weeks (3 weeks, not tested) of closing houses without doing anything; the house temperature was between approximately 1-3 degrees C. In broiler houses that have the possibility to be heated closed houses at 38 degrees C for 2 days will destroy the virus. Airborne routes of spread were not seen as very important and it is possible for adjacent houses to remain negative. The movement of people and equipment is seen as more important.

In summary, it is important to prevent contact between wild birds and affected poultry to prevent reassortment of avian influenza with the consequence of increasingly virulent strains arising. It is also important to monitor the genetic drift that occurs within poultry when there are outbreaks of avian influenza.

## REFERENCES

- Abolnik, C. et al., 2007. Outbreaks of avian influenza H6N2 viruses in chickens arose by a reassortment of H6N8 and H9N2 ostrich viruses. *Virus Genes*, 34(1), pp. 37-45.
- Abolnik C, de Castro M, Rees J. 2012. Full genomic sequence of an African avian paramyxovirus type 4 strain isolated from a wild duck. *Virus Genes*. 45(3), pp 537-541
- Advisory Committee on Dangerous Pathogens, 2008. *Risks to human health from non H5/7 Avian Influenza serotypes Risk Assessment of Low Pathogenicity H6 Avian Influenza to Humans in the UK*, s.l.: DEFRA.
- Australian Veterinary Emergency Plan, 2011. *Disease Strategy Avian Influenza Version 3.4*. [Online] Available at: <http://www.animalhealthaustralia.com.au> [Accessed 2 January 2013].
- European Commission Scientific Committee on Animal Health and Animal Welfare, 2000. *The definition of Avian Influenza. The use of vaccination against Avian Influenza*, s.l.: European Commission .
- Gillim-Ross, L. et al., 2008. Avian Influenza H6 Viruses Productively Infect and Cause Illness in Mice and Ferrets. *Journal of Virology*, 81(21), p. 10854–10863.
- Jackwood, M. W. et al., 2010. Biologic Characterization of Chicken-Derived H6N2 Low Pathogenic Avian Influenza Viruses in Chickens and Ducks. *Avian Diseases*, 54(1), pp. 120-125.
- Kim, H.-R. et al., 2010. Genetic relatedness of H6 subtype avian influenza viruses isolated from wild birds and domestic ducks in Korea and their pathogenicity in animals. *Journal of General Virology*, Volume 91, pp. 208-219.
- Kinde, H. et al., 2003. The Occurrence of Avian Influenza A Subtype H6N2 in Commercial Layer Flocks in Southern California (2000–02): Clinicopathological findings. *Avian Diseases*, 47(3), pp. 1214-1218.
- Li Q, Sun X, Li Z, Liu Y, Vavricka CJ, Qi J, Gao GF. 2012. Structural and functional characterization of neuraminidase-like molecule N10 derived from bat influenza A virus. *Proc Natl Acad Sci U S A*. 109(46), pp 18897-188902.
- Morales, A. C. et al., 2009. Biologic Characterization of H4, H6, and H9 Type Low Pathogenicity Avian Influenza Viruses from Wild Birds in Chickens and Turkeys. *Avian Diseases*, Volume 53, pp. 552-562.
- Nam, J.-H. et al., 2011. Emergence of Mammalian Species-Infectious and -Pathogenic Avian Influenza H6N5 Virus with No Evidence of Adaptation. *Journal of virology*, 85(24), p. 13271–13277.
- Ozaki, H. et al., 2011. Changing Patterns of H6 Influenza Viruses in Hong Kong Poultry Markets. *Influenza Research and Treatment*, Volume 2011, pp. 1-9.
- Swayne, D. E., 2003. Transcript of the Question and Answer Sessions from the Fifth International Symposium on Avian Influenza. *AVIAN DISEASES*, Volume 47, pp. 1219-1255.
- Swayne, D. E. et al., 2011. Assessment of national strategies for control of high-pathogenicity avian influenza and low-pathogenicity notifiable avian influenza in poultry, with emphasis on vaccines and vaccination. *Rev. sci. tech. Off. int. Epiz.*, 30(3), pp. 839-870.
- Webby, R. J., Woolcock, P. R., Krauss, S. L. & Webster, R. G., 2002. Reassortment and interspecies transmission of North American H6N2 influenza viruses.. *Virology*, 295(1), pp. 44-53.



# 3 TECH TOOLS FOR STATE AND PRIVATE VETERINARIANS

Henwood, V.C.<sup>1\*</sup> & Taylor, I.<sup>1</sup>

## INTRODUCTION

Computerised tools, applications and web based systems are going to become essential in the state veterinary services. This abstract is going to discuss the implementation of trails of three tools which could be available to state veterinary services. These three tools are namely the “live animal export certification system”, Sharepoint server 2010™ and Iauditor™

### *LIVE ANIMAL EXPORT CERTIFICATION SYSTEM*

The Live animal export certification system is a web based system with a SQL back end. The system involves an application from a person who wishes to export an animal. The applicant enters their details, the destination details and the animal details. The system then generates a report with a unique ID for the veterinarian.

The applicant takes the report to a veterinarian of their choice and the veterinarian ensures that the animal complies to the requirements as indicated by the system. This is done by entering all the required vaccination, test, treatment and general clinical details. The veterinarian then signs off on a system generated report.

The applicant then takes the report to the state veterinarian and the state veterinarian verifies that everything has been complied with. The state vet then generates the export certificate from the system.

The “Live animal export certification system” has the following advantages over previous systems:

1. speeds up applications for export certifications
2. ensures that client and veterinarians have the correct export certificates with the correct requirements
3. each and every animal’s unique identification, vaccinations, tests and treatments are typed on the report and therefore the system prevents tampering with the documents after they have been issued.
4. data from the system can be queried and reported to interested parties.

### *SHAREPOINT SERVER 2010™*

Sharepoint server is a user interface for a SQL database. It is a Microsoft product. The interface is customisable to whatever the user requires. Its main purpose is collaboration in the business environment. Access to the server is password protected. It is available online and on the internal network of the Western Cape Provincial Government.

In the Western Cape Department of Agriculture Share point is used for the following:

1. Standardisation of all export certificate and movement certificate templates across all of the provincial offices
2. Collaboration and discussion surrounding export issues
3. Management of the export establishment audit program
4. Calendar of all training and congresses available to state veterinarians
5. Contact details of all state veterinarians across South Africa is maintained by the user of Sharepoint
6. Development of SOP’s
7. Information to the certifying veterinarian regarding the production establishment for products to be exported.

### *IAUDITOR™*

Iauditor™ is an application developed by SafetyCulture an Australian company. The application is available on Android and Apple phones and tablets. Currently the application is being tested to see if it will be suitable for conducting export establishment audits. So far the advantages of Iauditor over current audit procedures:

1. Standardised templates for industry’s
2. Report generated on site
3. GPS location inputted by Iauditor
4. Signatures are taken electronically.
5. Photos of the non-conformances can be taken on site.
6. Photos taken can be annotated during the audit.
7. Audit reporting time is decreased by up to 50 %

<sup>1</sup> Private Bag X1, Elsenburg, 7607. 021 808 5056.

vincenth@elsenburg.com



## **CONTINUING EDUCATION PRESENTATION: AGRICULTURAL TRADE AS A DRIVER OF REGIONAL INTEGRATION IN THE SADC REGION- WHAT ARE THE OBSTACLES?**

Mulumba, M\*

### **ABSTRACT**

Agriculture is the major employer in the SADC region. The region as a whole has more small scale farmers compared to commercial farmers in a ratio of about 80:20. The livestock sub-sector makes up about 30-40 percent of the agriculture sector. Despite abundant and favourable natural and human resources, the region is a net importer of virtually all livestock products.

The stated aim of SADC as a regional block is Regional Integration that is to be achieved mainly through enhanced trade within the region. To this effect SADC declared a Free Trade Area (FTA) in 2008, which was to be followed by the Common Market in 2012. The date for the declaration of a Common market has been put back. The perception is that transboundary animal diseases (TADs) are the main obstacle to increasing trade in livestock and livestock commodities. Whether or not this is the major or indeed the only obstacle to increased intra SADC trade in livestock /livestock commodities is examined.

This paper makes a candid assessment of the region's intra and extra SADC trade in livestock/livestock products and suggests that more can be done to improve intra SADC trade. It is argued that with the region boasting some of the fastest growing economies in the world the rise of a middle class will result in an increased demand for protein and better prices for producers. On the other hand, indications are that current lucrative export markets for some countries will not be tenable for long. The major livestock producers should therefore target the region and the continent as these offer the best prospects for long term sustained profitable trade in livestock/livestock commodities.

# SERO PREVALENCE SURVEY OF *NEOSPORA CANINUM* IN COWS AND ITS ASSOCIATED RISK FACTORS IN UMUNGUNGUNDLOVU DISTRICT MUNICIPALITY

Chisi, L.C.<sup>a\*</sup>, Mbizeni, S.,<sup>b</sup> Perrett, K.,<sup>c</sup> Marageni, Y.,<sup>a</sup> Naidoo, P.,<sup>a</sup> & Zulu, G.<sup>a</sup>

## INTRODUCTION

*Neospora caninum* is an intra-cellular protozoa organism (Hall et al, 2005). *N. caninum* causes a disease called neosporosis in cattle (Coetzer and Tustin 2004). In the past it has been confused with *Toxoplasma gondii* (Dubey et al., 2007, Dubey et al., 1988) because it is structurally related to *Toxoplasma gondii*.

Even though *Neospora caninum* is very similar to *Toxoplasma gondii*, there are two key epidemiological aspects that differentiate the two diseases i.e.

1. Neosporosis primarily affects cattle and dogs and canines are the definitive hosts of *N. caninum*.
2. Toxoplasmosis affects humans, sheep and goats and felines are the only known definitive hosts.

Neosporosis is a serious disease of cattle and dogs. The major clinical sign of *N. caninum* infection in cattle is reproductive failure (Van Leeuwen, J.A., et al 2010). It is a major cause of abortions in beef and dairy cattle (Dubey et al., 1988). It can cause an abortion storm in a herd of cattle (Thornton, R.N. et al. 1994). Economic losses i.e. decreased milk yield, premature culling and indirect veterinary costs such as private vet costs and diagnostic costs are also major consequences of *N. caninum* infection on a farm.

In dogs it manifests mainly as a neuromuscular disease. (Hall et al, 2005). It can also result in still births and neonatal mortalities.

## METHODS

This study was conducted in uMgungundlovu District Municipality (UDM) of KwaZulu Natal province of South Africa between March 2012 and May 2012. It was a cross sectional study. Sample size was 331 cows and a two stage cluster design was used to sample animals. Three practices i.e. Howick, Mooi River, Vet House and State Vet Pietermaritzburg offices participated in the survey.

## RESULTS

The crude sero prevalence of *Neospora caninum* in UDM was 21.2% (95% CI: 16.9-25.9). The sero prevalence of the individual municipalities was as follows, Impendle 26.1% (CI 95%:14.3-41.1), Mooi Mpofana 19.6 (CI 95%:13.6-26.8), uMgeni 37.3% (CI 95%:25.0-50.8) and Msunduzi / Richmond/uMshwathi 8.2% (CI 95%: 3.1-17.0). The crude prevalence in dairy cows was 26.82 % (95% CI, 23.72%-36.36%), whilst that for beef was 5.17% (95% CI, 1.92%-10.91%).

## CONCLUSION

This study established that the sero prevalence of *Neospora caninum* in cows was 21.2% and that not culling sero positive animals was a risk factor. Animals under the authority of SVPMB were unlikely to be sero positive for *Neospora caninum* compared to those under the watch of the three private vet clinics. It is recommended that *Neospora caninum* be included in the differential diagnosis for abortion cases on farms. It is further recommended that testing of cows for *Neospora caninum* on farms be considered as part of reproductive management.

<sup>a</sup> Allerton Provincial Veterinary Laboratory, Pietermaritzburg, KwaZulu Natal, South Africa.

<sup>b</sup> Pietermaritzburg (uMgungundlovu) State Veterinarian,

<sup>c</sup> Epidemiology Department of Agriculture, Private Bag X2, Cascades 3202, KwaZulu Natal, South Africa.

\* Corresponding author

Tel :+27 33 347 6284 ,

email: [Songelwayo.CHISI@kzndae.gov.za](mailto:Songelwayo.CHISI@kzndae.gov.za) ,

twitter: @docsonge,

Address : Private Bag X2, Cascades, 3202 ,

## REFERENCES

- Bartels, C.J., Wouda, W., Schukken, Y.H. 1999. Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). *Theriogenology* 52, 247–257.
- Coetzer, J.A.W & Tustin, R.C. 2004. *Infectious Diseases of Livestock*. Volume I, pages 382-393.
- Dijkstra, T., Barkema, H.W., Eysker, M., Beiboer, M.L., Wouda, W., 2003. Evaluation of a single serological screening of dairy herds for *Neospora caninum* antibodies. *Veterinary Parasitology* 110, 161–169.
- Dohoo, I., Martin, W. & Stryhn T, H. 2010. *Veterinary Epidemiologic Research*, Pei, Ver Inc.
- Dubey, J.P., Carpenter, J. I., Speer, C. A., Topper, M. J. & Uggla, A. 1988. Newly recognized fatal protozoan disease of dogs. *Journal of American Medical Veterinary Association*, 192, 1269-1285.
- Dubey, J.P., Schares, G. & Ortega-Mora, L. M. 2007. Epidemiology and Control of Neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews*, 20, 323-327.
- Hall, C.A. Reichel, M.P., Ellis, J.T. 2005. *Neospora* abortions in dairy cattle: diagnosis, mode of transmission and control. *Veterinary Parasitology* 128, 231–241.
- Hemphill, A. & Gottstein, B., 2000. A European perspective on *Neospora caninum*. *International Journal of Parasitology*. 30, 877–924.
- Hobson, J.C., Duffield, T.F., Kelton, D., Lissemore, K., Hietala, S.K., Leslie, K.E., McEwen, B., Peregrine, A.S., 2005. Risk factors associated with *Neospora caninum* abortion in Ontario Holstein dairy herds. *Veterinary Parasitology* 127, 177–188.
- IDEXX 2010. *IDEXX Neospora X2*. Westbrook, Maine.
- Loobuyck, M Frossling, J., Lindberg, A., Bjokman, C. 2009. Seroprevalence and spatial distribution of *Neospora caninum* in a population of beef cattle. *Preventive Veterinary Medicine* 92: 116-122
- Reichel, M.P., 2000. *Neospora caninum* infections in Australia and New Zealand. *Australian Veterinary Journal*. 78, 258–261.
- Thornton, R.N., Gajadhar, A., Evans, J., 1994. *Neospora* abortion epidemic in a dairy herd. *New Zealand Veterinary Journal*. 42, 190–191.
- VanLeeuwen, J.A., Haddad, J.P., Dohoo, I.R., Keefe, G.P., Tiwari, A. Scott, H.M. 2010. Risk factors associated with *Neospora caninum* seropositivity in randomly sampled, Canadian dairy cows and herds. *Preventive Veterinary Medicine* 93:129–138.

# ARTICLE PUBLISHED

NJIRO, S.M., KIDANEMARIAM, A.G., TSOTETSI, A.M., KATSANDE, T.C., MNISI, M., LUBISI, B.A., POTTS, A.D., BALOYI, F., MOYO, G., MPOFU, J., KALAKE, A. & WILLIAMS, R., 2011, 'A STUDY OF SOME INFECTIOUS CAUSES OF REPRODUCTIVE DISORDERS IN CATTLE OWNED BY RESOURCE-POOR FARMERS IN GAUTENG PROVINCE, SOUTH AFRICA', J.S.AFR.VET.ASSOC. 82(4), 213-218.

## A STUDY OF SOME INFECTIOUS CAUSES OF REPRODUCTIVE DISORDERS IN CATTLE OWNED BY RESOURCE-POOR FARMERS IN GAUTENG PROVINCE, SOUTH AFRICA

Njiro, S.M., Kidanemariam, A.G., Tsotetsi, A.M., Katsande\*, T.C., Mnisi, M., Lubisi, B.A., Potts, A.D., Baloyi, F., Moyo, G., Mpofu, J., Kalake, A. & Williams, R.

### ABSTRACT FROM PUBLISHED ARTICLE

Two hundred and thirty-nine cattle from Gauteng Province in South Africa were tested for various pathogens causing reproductive diseases including bovine viral diarrhoea/mucosal disease (BVD/MD) virus, infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus, *Neospora caninum* and *Brucella abortus* using various tests. For BVD/MD virus, 49.37% tested positive, 74.47% for IBR/IPV virus, 8.96% for *Neospora caninum* and 3.8% for *Brucella abortus*. The result for *Brucella abortus* is higher than the national average, possibly due to the small sample size. A high seroprevalence of antibodies to both BVD/MD virus and IBR/IPV virus was evident. These 2 viruses should be considered, in addition to *Brucella abortus*, when trying to establish causes of abortion in cattle. The clinical significance of *Neospora caninum* as a cause of abortion in Gauteng needs further investigation. One hundred and forty-three bulls were tested for *Campylobacter fetus* and *Trichomonas fetus*, and a low prevalence of 1.4% and 2.1% respectively was found in this study. The clinical implications of these findings are discussed

# ARTICLE PUBLISHED

TSOTETSI, A.M., NJIRO, S., KATSANDE, T.C., MOYO, G., BALOYI, F. & MPOFU, J., 2013, 'PREVALENCE OF GASTROINTESTINAL HELMINTHS AND ANTHELMINTIC RESISTANCE ON SMALL-SCALE FARMS IN GAUTENG PROVINCE, SOUTH AFRICA', *TROP.ANIM HEALTH PROD.* 45(3), 751-761.

## PREVALENCE OF GASTROINTESTINAL HELMINTHS AND ANTHELMINTIC RESISTANCE ON SMALL-SCALE FARMS IN GAUTENG PROVINCE, SOUTH AFRICA

Tsotetsi, A.M., Njiro, S., Katsande\*, T.C., Moyo, G., Baloyi, F. & Mpofo, J.

### ABSTRACT FROM PUBLISHED ARTICLE

The present study was conducted to determine the prevalence and distribution of gastrointestinal helminths, to detect the presence of anthelmintic resistance in livestock from small-scale farms and to determine the level of helminthosis awareness among small-scale farmers in Gauteng Province, South Africa. Blood and faecal samples were collected from cattle (n = 314), sheep (n = 256) and goats (n = 311). Faecal egg counts and cultures were done, helminth genera identified and packed cell volume was assessed to detect anaemia. A faecal egg count reduction test was used to determine anthelmintic resistance against albendazole (7.5 mg/kg), levamisole (5 mg/kg) and ivermectin (0.2 mg/kg) on five small ruminant farms. A high prevalence of both nematodes and trematodes was observed; however, only 1 % of cattle had high nematode egg counts compared to goats (30 %) and sheep (32 %). Only 5 % of the animals were anaemic. *Haemonchus* and *Calicophoron* were the most dominant helminth genera in the studied ruminants. Anthelmintic resistance was detected against the three tested drugs on all the screened farms, except against albendazole and levamisole in sheep from Hammanskraal and Nigel, respectively. About 88 % of interviewed farmers were aware of veterinary helminthosis, 67 % treated against helminths and 83 % provided their livestock with nutritional supplements. This study showed that a high prevalence of helminthosis and anthelmintic resistance does occur in the study area, thus relevant strategic interventions are recommended

\*Presenting author

# **PARTICIPATORY METHODS TO CHANGE PERCEIVED RISKS AND INCREASE UPTAKE OF DISEASE PREVENTIVE MEASURES**

Woods, P.S.A\*, & Taylor, N.M.

## **ABSTRACT**

In Zimbabwe, most small-scale communities were free from brucellosis and unaware of it. However, finances constrained the veterinary services and uncontrolled cattle movements after farm resettlements introduced *Brucella*-positive cattle into naive, unvaccinated areas.

We hypothesized that informing farmers about the dangers of Contagious Abortion (CA) to their families, and working with these communities to decide possible management options for positive cows, would alter their perceived risk of brucellosis to their cattle and families and increase the uptake of preventive measures. The emphasis was on “perceived risk of zoonotic brucellosis”, rather than “level of knowledge about brucellosis” as we considered that the risk would be the motivating factor for taking appropriate action.

Working with the small-scale dairy cooperative network, veterinary and extension services, nurses, community health workers and local leaders, we developed short courses about brucellosis, its epidemiology and developed community action plans to prevent *Brucella* entering an area or infecting people/cattle. The emphases were varied to audiences and their future roles in surveillance and detection. According to each group’s discussion, subsequent interventions were conducted.

Repeated structured interviews at 120 households compared brucellosis knowledge levels, cattle management and milk handling practices. The percentage of farmers with an “excellent” knowledge about CA increased from just over 50% in the project’s second year (2009) to over 80% in 2011.

The relationship of the risk perception “Are your cattle susceptible to CA?” to a calculated “Farmer’s CA knowledge index” varied depending on whether there had been a cattle abortion on the farm in the last 24 months ( $n=302$ ). If no abortion had occurred then  $z=8.05$   $p<0.01$ , whereas if an abortion had occurred then  $z=45.142$   $p<0.001$ . The relationship between knowledge index and the risk perception of “Is your family susceptible to Brucellosis?” also varied, and was only significant if abortions had occurred  $z=15.167$   $p<0.001$ . This perception of human risk was also related to the preventive practice of S19 calf vaccination and varied depending whether there had been abortions on the farm.

It is recommended that in future surveys examining livestock keeper’s behavioural changes, that specific questions about the respondent’s perceptions be explicitly asked rather than assumed as it is how that person feels and reasons which will influence their future decisions and behaviours.

\*Contact Author:

Department of Animal Science, Faculty of Agriculture, University of Zimbabwe, PO Box MP 167, Mt Pleasant, Harare, Zimbabwe

Phone +263 773 236 152

Email: pwoods2@hotmail.com

# PERCEPTION OF COMMUNAL FARMERS ON FOOT AND MOUTH DISEASE CONTROL AT THE WILDLIFE/LIVESTOCK INTERFACE OF THE KRUGER NATIONAL PARK

Lazarus, D.D.<sup>1,5\*</sup>, Burroughs, R.<sup>1</sup>, van Schalkwyk, O.L.V.<sup>2</sup>, Mpehle, A.<sup>3</sup>, Thomson, G.R.<sup>4</sup> & Fosgate, G.T.<sup>1</sup>

## ABSTRACT

Areas surrounding the Kruger National Park (KNP) are part of the Foot-and-mouth disease (FMD) control zone with the KNP being an infected area due to the presence of African buffalo (*Syncerus caffer*), which serve as the major reservoir to the South African Territories (SAT) serotype of FMD viruses. The aim of this study was to evaluate the perceptions of communal farmers concerning the current FMD control interventions at the wildlife/livestock interface.

A structured questionnaire was administered to farmers as they presented their cattle at dip tanks within the Mnisi Tribal Area. The questionnaire was applied through in-person interviews using the local language (Shangaan). Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology. Two hundred and eighty-six cattle were independently selected to estimate the proportion with high titres against FMDV-structural proteins; this was assumed to indicate an immunological response to routinely administered vaccines because of the absence of recently recorded outbreaks.

One hundred and four farmers responded to the questionnaire with 73% (76/104) being cattle owners with the remainder being hired cattle handlers. The majority of respondents, (79%; 95% confidence interval, 70% - 80%) indicated a high level of satisfaction with the current animal health programmes at the dip tank. The educational level of farmers varied over levels of satisfaction with the median education level being standard 9 (IQR: 2 – 12) for non-satisfied respondents, standard 3 (IQR: 0 – 6) for little satisfied and standard 7 (IQR: 2 – 11) for very satisfied respondents ( $P = 0.036$ ). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance ( $P = 0.002$ ). The majority of respondents identified the African buffalo as a risk for FMD outbreaks (92%, 95% confidence interval: 85% - 96%).

Relative to an antibody titre of  $\geq 1.6 \text{ Log}_{10}$  (1:40 dilution), 20% (95% confidence interval: 15% - 27%) of sampled cattle had serologically converted to SAT 1, 38% (95% confidence interval: 32% - 45%) to SAT 2 and 24% (confidence interval: 18% – 31%) to SAT 3. This indicates an unsatisfactory immune response to the current vaccination schedule (six-monthly mass vaccination) and therefore that the level of satisfaction expressed by cattle owners in the animal health programme may be misplaced, at least as related to FMD control.

1 University of Pretoria, Faculty of Veterinary Science;

2 Office of the State Veterinarian, Kruger National Park;

3 Mpumalanga Veterinary Services;

4 TADScientific, Pretoria;

5 National Veterinary Research Institute, Vom Nigeria.

\*Contact: +27 74 634 7529, lazdav2003@yahoo.co.uk

# **PARTICIPATORY APPRAISAL OF PESTE DES PETITE RUMINANTS (PPR) OUTBREAKS IN ISEYIN LOCAL GOVERNMENT AREA OF OYO STATE, NIGERIA**

Morakinyo O. A<sup>1,2</sup>. & Babalobi O.O<sup>1</sup>

## **SUMMARY**

Following informal reports to the Oyo State Coordinator Veterinary Epidemiology unit, of ongoing suspected outbreak of Peste des Petit Ruminants (PPR) by community members from Iseyin, a major sub-urban town, northwest Oyo State, Southwest Nigeria, (where about 80% of rural families especially women and children keep sheep and goats); a Participatory Appraisal approach (that enable local people to identify their own (animal health) problems and make decisions on possible solutions), was applied to ascertain the livestock farmer's awareness and perspective of the importance of PPR.

Respondents symptomatically identified PPR (*aayo/lukuluku ewure/Ayohere*), Diarrhoea (*igbegburu*), Mange (*aami/fofo/ ekuru*) and Tetanus (*agan*), as the major diseases and health problems in the community, with PPR identified as the major disease by almost all the respondents, using different symptomatic names for the disease. There was a considerable awareness of the disease amongst the farmers and goat sellers who had experienced outbreaks of the disease. Most of them were unaware of the disease until they experienced outbreaks in their flocks. There was little awareness of preventive measure (including vaccination), just as there were practically non-existent public health care delivery to the farmers, with only one private veterinarian and a lot of unregulated quacks operating freely in the community. Thus outbreaks of PPR in the study area go virtually unreported.

The present surveillance system does not have the sufficient capacity to detect the disease early enough and most times, little or no efforts are made to control the disease. A proper understanding and application of the Participatory Appraisal and other Participatory Epizootiology (Veterinary Epidemiology) approaches will significantly improve Nigeria's animal healthcare service delivery and surveillance systems for the largely rural based livestock holders and ultimately lead to improved animal population health in Nigeria.

## **INTRODUCTION**

Livestock production remains very important to the people of Africa. In almost all countries, it's a major source of government revenue and export earnings. It is the source of livelihood to many as it sustains the employment and income of millions of people in Africa, particularly people in the rural areas who derive their entire livelihoods from Livestock farming. (Brumby, 1990) In many African countries, small ruminants (sheep and goats) constitute a substantial proportion of the nation's meat supply. For women and children in particular, small ruminants are the major livestock that they keep and it provides an additional source of income to them or in some cases, the only source of income. Small ruminants' production is faced with a lot of constraints including poor nutrition, scarce or unavailable food products and poor management practices. However, the most important constraint to small ruminant production is PPR.

Peste des Petits Ruminants (PPR) is considered the most important single cause of morbidity and mortality for sheep and goats, in Africa. Peste des Petits ruminants (PPR) is a highly contagious and infectious viral disease of domestic and wild small ruminants. Peste des Petits ruminants is a disease of major economic importance. It is regarded as the biggest constraint to large- scale intensive production of sheep and goats in the West African sub-region. It is acknowledged as the most destructive disease and the number one killer disease of small ruminants in West Africa. In susceptible flocks, morbidity may be 100% and mortality greater than 90 %, especially amongst animals under six months of age. Even though there is an effective vaccine for the cure of this disease. However, outbreaks of the disease keep occurring yearly

Participatory epizootiology (PE) (Mariner et al, 2000) is based on conventional epizootiological concepts but uses participatory methods (Chambers, 1983; McCracken et al, 1988) to solve epizootiological problems. It is a practical approach to epizootiological studies that gives stakeholders a greater role in shaping programmes for public health (Loewenson, 2004), animal health, disease surveillance, research. The techniques of participatory rural appraisal (PRA) are used to formulate the programme objectives, gather epizootiological data and intelligence, and analyse information. Participatory epizootiology recognizes that local people have very rich and detailed knowledge about the animals they keep and the infectious and zoonotic diseases that can gravely affect their livelihoods and endanger human health. Local farmers and livestock owners are often able to describe clinical presentations, epizootiological patterns and principal pathological lesions using a vocabulary of specific

<sup>1</sup>Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

<sup>2</sup>Livestock Improvement Program,

Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria

E-mail address: [daremorakinyo@gmail.com](mailto:daremorakinyo@gmail.com)

Phone: +234-8056131695



disease terms in local languages that correspond to Western clinical case definitions. This body of knowledge has been termed 'existing veterinary knowledge' (EVK) (Mariner et al, 2000). Participatory epizootiological learns from local knowledge, leading to disease control programmes that are both acceptable to their stakeholders and effective. The PE approach was developed to overcome the constraints in applying conventional epizootiology and formal research in developing countries.

Participatory approaches were made use of in this study to ascertain the farmer's awareness of PPR and their perspective of the importance of the disease in their farming operations. It was also used to determine if there was any form animal healthcare delivery and to what extent it was

## MATERIALS AND METHODS

The study was conducted in Iseyin which is located about 100km Northwest of Ibadan, the state capital of Oyo State. Iseyin is bounded by Latitude 7°57'N & 8°17'N and longitude 2°45'E & 3°37'E. Iseyin covers an estimated landmass of 1,341.766 square kilometres and an estimated population of 291,310 (2010 estimate). Iseyin was selected as the study site because of informal reports to the state coordinator of the epidemiology unit of the State ministry of Agriculture and Natural Resources, from some community members about an ongoing outbreak of PPR in the town. A team was formulated which comprised of three veterinarians. A pilot study was carried out to have an overview of the community. This includes identification of the key informants and entry points.

Participatory appraisal methods were applied to generate information on PPR in Iseyin. The participatory appraisal methods used were interviewing methods which employed the use of Semi-structured interviews (SSI). The tools made use of include Key Informant interviews, Focus group discussions and individual interviews.

Focus group discussions were conducted with three categories of respondents viz; goat sellers (20), peri-urban goat farmers (6) and rural goat farmers (Fulani) (6). In addition, individual interviews were conducted with four people viz: a private veterinarian, the head of the Goat sellers, a peri-urban and a rural goat farmer. Other materials that were used includes, 10.1 megapixels digital camera, voice recorder, customary gifts for community members and honoraria, stationery and writing materials

## RESULTS

### SOURCE OF LIVELIHOOD

Key informant interviews, focus group discussions and even individual interviews revealed that all respondents were involved with goats directly or indirectly. However, not all of them derive their livelihood entirely from goat production.

The goat sellers are dependent on the sale of goats for their survival. Most of the peri-urban goat farmers are not dependent on goat production for their survival. A lot of them have other sources of livelihood and goat production is just an adjunct. However, the rural goat farmers are dependent on goat production for their survival. The importance of Goat production to the livelihoods of the respondent is described as the degree to which they depend on Goat production for their survival. The relative importance of goat production to their livelihoods is described in the table below.

Also, the study revealed that more women are involved with goat production than men. Amongst the Fulani pastoralists, the women and the children are the ones involved with goat production while the men are involved with farming and cattle rearing. However, both men and women are equally involved with the sales of goats.

**Table 1: Relative importance of goat production to the livelihoods of respondents**

Category	Relative importance of goat production to livelihoods
Goat sellers	Very important
Peri-urban goat farmers	Quite important
Rural goat farmers	Very important

### HEALTH PROBLEMS

The respondents were able to identify a number of diseases and health issues that poses a constraint to goat production among which are *igbegburu* (Diarrhea), *aami*, *fofo*, *ekuru* (Mange), *aayo* (PPR), *agan* (Tetanus), etc. However, the most important disease that kept recurring from almost all the respondents was PPR. It is called by different names amongst the respondents such as *lukuluku ewure*, *aayo*, *ayohere*, etc.



Figure 1: A focus group discussion with goat sellers

### PPR AWARENESS

There is a considerable awareness of the disease amongst the farmers and goat sellers. This is mainly associated with their past experiences with the outbreak of the disease. Most of them were unaware of the disease until they experienced outbreaks in their flocks. It was only the Fulani rural farmers who claimed they were unaware of the disease. This may be due to the language barrier that hindered effective communication.

### PPR IMPORTANCE

It is generally agreed amongst all those who have experienced outbreaks of PPR that the disease is the most important disease affecting their goats. Even though they mentioned a number of different diseases, they all agree that PPR is the most important of them all.



Figure2: Informal interview with an identified Fulani Goat farmer.

### ETHNOVETERINARY KNOWLEDGE

There is presently no existing veterinary knowledge for the management and control of the disease amongst the farmers. Usually, when an outbreak occurs, farmers just watch helplessly as their animals die in their numbers

### PPR CONTROL

There is no method of control of the disease being practiced amongst the farmers. The only effective means of control is by vaccination which most of the farmers are not aware of. Also, the farmers are ignorant of the pattern of the disease and so they don't know what specific precautionary measures to take for the control of the disease.



Figure 3: Focus group discussion with Fulani men

### HEALTHCARE DELIVERY

There is a practically non-existent health care delivery to the farmers; the only form of health care delivery that they receive is from the private veterinarian in the community and then a lot of quacks who are operating freely in the community without any form of supervision or training.

### CONCLUSION

The control of PPR therefore should be of utmost importance to Nigeria as a country seeing the enormous socio-economic significance of the disease, not only to individual farmers but to the country as a whole. An effective control of the disease in Nigeria is going to significantly improve the economy of the country and the livelihoods of this rural people. It will also improve our International trade in Goat and Goat products.

The control of PPR in Nigeria is the responsibility of Nigeria's Veterinary Service which falls under the Federal Department of Livestock and Pest Control Services (FDL&PCS) of the Ministry of Agriculture and Water Resources.

Inasmuch as there seems to be an efficient structure that allows for surveillance, reporting and control of these TADs including PPR, there are still a lot of constraints to its operation. A lot of times, outbreaks of PPR go unreported. The surveillance system does not have the actual capacity to detect the disease early enough and most times, little or no efforts are made to control the disease. From time to time, the government attempts to organize mass PPR vaccination campaigns but most times the efforts prove abortive as either the campaigns eventually do not hold or they happen at a time when the outbreak has already started thus, being counterproductive.

The effective control of PPR is going to demand an effective surveillance and disease control system. The most effective means for the control of the disease is by vaccination. There is a homologous PPR vaccine which can confer an immunity of up to 3 years.

### RECOMMENDATIONS

Adoption of Participatory epizootiology by Nigeria's veterinary service is going to help increase the efficiency of veterinary service delivery in Nigeria. Participatory epizootiology is the application of participatory methods to epizootiological research and disease surveillance. It is a proven technique which overcomes many of the limitations of conventional epizootiological methods. One of the branches of PE, which is Participatory Disease Search, is an important tool that has been used as a form of active surveillance in other parts of Africa. It has proven very effective especially in the eradication of Rinderpest in the horn of Africa. PDS was used to identify current foci of Rinderpest outbreaks (Mariner, 1996).

Secondly: Community Based Animal Health Systems will particularly help to improve veterinary service delivery especially in rural and remote areas. It is a form of primary animal health care delivery that makes use of Community Animal Health Workers (CAHWs). These are community members that are chosen from amongst the community and are trained in the basic principles of animal health care delivery and are then released to work in their various communities under an expert supervision of either a Veterinarian or an Animal Health Technologist. These CAHWs have been made use of in several African countries with tremendous success in such countries. An assessment of the impact of CAHWs revealed that they have significantly contributed to improving human livelihoods, epizootic disease control and also improving disease surveillance and reporting systems (Leyland and Catley, 2002). CAHW systems are the most economically efficient way to provide privatised veterinary services (Leyland and Catley, 2002). CAHWs have been used as Vaccinators in Somaliland where they achieved 95% vaccination efficiency, one of the highest that has ever been recorded in Africa (Mariner et al, 1994).

## REFERENCES

- Ademosun, A. A. (1985), Contributions of research to small ruminants production in Nigeria. In: Adu, I. F.; Osinowo, O. A.; Taiwo, B. B. A. & Alhassan, W. S. (Eds). Small ruminant production in Nigeria. Proceedings of National Conference on Small Ruminant Production held in Zaria, Nigeria, 6-10 October 1985. Shika, NAPRI (National Animal Production Research Institute),
- Bourdin P. 1983. History, epidemiology and economic significance of PPR in West Africa and Nigeria in particular. In: Hill D H (ed), Peste des Petits ruminants (PPR) in sheep and goats. Proceedings of the international workshop held at IITA, Ibadan, Nigeria, 24-26 September 1980. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia. pp. 10-11
- Brumby P.(1990). Systems and livestock development in tropical Africa. In: Dauda K Jawara (ed), Animal disease as a factor limiting economic development in Africa .Comell Veterinary.80:17-25.
- Chambers, R. (1983). Rural Development: Putting the Last First. Longman Scientific and Technical, New York
- Diallo A (2006). Control of Peste des Petits Ruminants and Poverty Alleviation? J. Vet. Med. B 53, 11–13
- Hamdy, F. M., A. H. Dardiri, D. Nduaka, S. S. Breese, and E. C. Ihemelandu, 1976: Etiology of stomatitis pneumocomplex in Nigeria dwarf goats. Can. J. Comp. Med. 40, 276–284.
- Jaitner, J.; Sowe, J.; Secka-Njie, E. & Dempfle, L. Ownership pattern and management practices of small ruminants in the Gambia - implications for a breeding programme. Small Rum. Res., 40:101-8, 2001.
- Kumar, S.; Vihan, V. S. & Deoghare, P. R. Economic implication of diseases in goats in India with references to implementation of a health plan calendar. Small Rum. Res., 47:159-64, 2003.
- Leyland T and Catley A (2002): Community-Based Animal Health Delivery Systems: Improving The Quality Of Veterinary Service Delivery; Paper prepared for the OIE Seminar Organisation of Veterinary Services and Food Safety World Veterinary Congress, Tunis, September 2002
- Loewenson R. (2004). – Epidemiology in the era of globalization: skills transfer or new skills? Int. J. Epidemiol., 33 (5), 1144-1150. Epub.: 6 May 2004.
- Majiyagbe K A. (1985). Health management in small ruminants with emphasis on Peste des Petits ruminants (PPR). In: Adu I F. Osinowo O A, Taiwo B A and Alhassan W S (eds), Small ruminant production in Nigeria. Proceedings of National Conference on Small Ruminant Production held in Zaria, Nigeria, 6-10 October 1985. NAPRI (National Animal Production Research Institute), Shika, Zaria, Nigeria. pp. 22-230
- Mariner, J.C. (1996). The World Without Rinderpest: Outreach to Marginalised Communities. In: The World Without Rinderpest. FAO Animal Health and Production Paper 129, 97-107.
- Mariner, J.C., Akabwai, D., Leyland, T.J., Lefevre, P.C. and, Masiga, W.N., (1994). Strategy for the Eradication of Rinderpest from Africa with Thermostable Vero Cell-adapted Rinderpest Vaccine. Proceedings of the International Symposium on Morbillivirus Infections. Hannover Veterinary School, Germany, 12-13 June 1994.
- Mariner, J.C., Paskin, R., (2000). Manual on Participatory Epidemiology. FAO Animal Health Manual 10. Food and Agriculture Organization, Rome.
- McCracken J., Pretty J. & Conway G. (1988). – An introduction to rapid rural appraisal for agricultural development. Institute for International Environment and Development, London.
- Rowland, A.C. and Bourdin, A. 1970. The histological relationship between Peste des Petits ruminants and Kata in West Africa. Rev. Elev. Méd. Vét. Pays Trop. 23 (3) : 301. 307.
- Rowland, A.C., Scott, G.R. and Ramachandran, S. 1971. A comparative study of Peste des Petits ruminants and Kata in West African dwarf goats. Anim. Health Prod.3(4) : 241. 247.
- Whitney, J.C., Scott, G.R. and Hill, D.H. 1967. Preliminary observations on stomatitis and enteritis of goats in southern Nigeria. Bull. Epizoot. Dis. Afr. 15:31. 41.

# MASS PET STERILISATION INTERVENTIONS IN INDIGENT COMMUNITIES IN THE BOLAND: A LESSON IN VARIATION

van Helden, L. S.\*

## INTRODUCTION

In early 2011, the Western Cape Department of Agriculture put aside R500 000 to fund a mass pet sterilization pilot project, aimed at creating a prototype for pet sterilization projects to be rolled out in the Western Cape in the future. Collaborating with the South African Veterinary Association, the Cape Animal Welfare Forum and Community Veterinary Clinics, the township of Kayamandi was chosen as the location for the pilot project to take place. It was estimated that there were approximately 3000 pets in the township, which, at R250 per sterilization, would allow all the funds to be used to sterilize 70% of the pet population. Furthermore, Kayamandi is a relatively isolated area, surrounded mostly by farmland and industrial areas of Stellenbosch, and it was envisaged that the pet population was therefore quite discrete and the effects of a pilot project could be measured relatively easily.

## MATERIALS AND METHODS

A full pet census was performed in Kayamandi by animal health technicians, volunteers from welfare organisations and volunteers from the local community going door to door recording details and basic health data of the pets living at each address. The initial census in Kayamandi revealed that there were only 1034 pets in the township; much fewer than expected. The sterilization phase lasted for six months, the goal being to sterilize 70% of the population of cats and dogs respectively within this period. The project steering committee subsequently decided, due to the excess funds that had not been used, to extend the project to Klappmuts, a small rural town 16km north of Stellenbosch. Again, it was chosen as it is a relatively isolated community estimated to be of the correct size for utilization of the remainder of the funds. A census performed in Klappmuts in December 2012 revealed 1200 pets and the sterilization phase lasted from February 2013 until June 2013. Funds were allocated to be used for sterilization costs only. Peripheral costs were funded by donors including the local municipality, the local animal welfare organization and Community Veterinary Clinics as well as numerous private donors. A follow-up census was performed in Kayamandi in April 2013, as part of a follow-up programme that will be followed in both project locations: follow-up census will be performed six months after the start of the sterilization phase, one year after the start of the sterilization phase and again two years after the start of the sterilization phase.

## RESULTS

The long term success of the project cannot be evaluated at this stage, as the follow-up phase is still to be completed. However using preliminary census data, several insights into the pet population composition in the communities were gained. These figures will be discussed in the presentation.

	Kayamandi	Klappmuts
<b>People: Pets</b>	25:1	7:1
<b>Dogs: Cats</b>	70:30	71:29
<b>Male: Female (dogs)</b>	62:38	58:42
<b>Male: Female (cats)</b>	42:58	43:57
<b>Percentage sterilized (dogs)</b>	26%	18%
<b>Percentage sterilized (cats)</b>	16%	13%
<b>Percentage adult named (dogs)</b>	81%	98%
<b>Percentage adult named (cats)</b>	61% (21%)	80% (35%)

Table 1: Comparison of aspects of pet population composition in Kayamandi and Klappmuts

Subjectively, the most significant difference observed between the two communities was enthusiasm for the project. While in Kayamandi, the temporary clinic that was set up stood empty every day and volunteers had to go door to door to source animals to sterilize, in Klappmuts a constant queue of people waited outside the sterilization clinic. Several community members of Klappmuts offered their services as volunteers at the clinic, handling the animals and educating visitors, while another community member organized a “dog show,” open to all the people of Klappmuts to encourage them to take pride in and take care of their pets.

While R250 was provided per animal sterilized by the WCDOA, the calculated total cost per animal was closer to R550. This included peripheral expenses such as microchips, deworming and vaccines, as well as salaries of welfare workers, transport and education in the community (Roos, A, 2013). For these projects some of these costs were not a factor, as, for example, volunteers were used instead of salaried workers and donated vaccines were sourced. However, in order to make future projects sustainable and not reliant on donations, these extra costs must be considered.

At the end of the sterilization phases in Klapmuts and Kayamandi, preliminary data indicates that the 70% sterilization targets were met.

	Kayamandi		Klapmuts	
	Dogs	Cats	Dogs	Cats
<b>Percentage sterilised</b>	81%	82%	79%	112%

**Table 2: Number of sterilized animals in the pet population at the end of the sterilization phase expressed as a percentage of the total animals counted in the initial census**

## DISCUSSION

There are currently no international guidelines available regarding what percentage of a pet population should be sterilized in order to maintain a stable population size (Totton, et al., 2010). A 70% sterilization rate was used in this case as it is the percentage of the pet population recommended by the World Health Organisation to be vaccinated against rabies to control the disease in the population (WHO, 2004). As the sterilization projects took place in conjunction with mass rabies vaccination campaigns, the two goals were combined.

The collection of data during the census and sterilization phases of the project was one of the biggest challenges. Numerous volunteers, welfare workers and animal health technicians gathered data, but as many of the measures were relatively subjective, such as body condition score and estimated age, interpretation of these variables was challenging. Even basic data such as the species and sex of the pet was often not directly measurable as the pet was not at home at the time of census and the owner provided all information. Insufficient record keeping was also a problem. In Klapmuts, development of the town has boomed: in 2012, 99 RDP houses were built, followed by another 380 in the first half of 2013. Concurrently, private developers built an unknown number of houses (Stellenbosch Municipality, 2013). The influx of new residents caused an increase in the pet population as they brought pets along with them. This created difficulty as the number of sterilisations required to sterilize 70% of the pet population became unknown. This effect is clearly seen in Table 2, in which more cats were sterilized than the total population counted during the census.

It has been observed that in different countries the demographics of the pet population and attitudes towards intervention campaigns differ greatly (Davlin and Von Ville, 2012). This raises many questions as to how much research must be done on the culture and composition of a community before a successful pet sterilisation intervention can take place, as well as how one can ensure community participation in such an initiative.

## REFERENCES

- Davlin, S.L. and Von Ville, H.M., 2012. Canine rabies vaccination and domestic dog population characteristics in the developing world: a systemic review. *Vaccine*, 30, pp.3492-3502.
- Roos, A., 2013. *Budget-Kayamandi pilot project*. [email](Personal communication, 7 Feb 2013).
- Stellenbosch Municipality, 2013. *Discussion regarding building of RDP houses in Klapmuts*. [telephone conversation] (Personal communication, 12 June 2013).
- Totton, S.C., Wandeler, A.I., Zinsstag, J., Bauch, C.T., Ribble, C.S., Rosatte, R.C. and McEwen, S.A., 2010. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. *Preventive Veterinary Medicine*, 97, pp.51-57.
- World Health Organisation, 2004. *WHO expert consultation on rabies: first report*. Geneva: World Health Organisation.

## **PREVENTION OF COMMUNICABLE DISEASES: THE HUMAN HEALTH PERSPECTIVE**

Blumberg, L.H.\*

### **ABSTRACT**

With respect to the conference theme of disease prevention in veterinary medicine, one can consider 3 different 'interpretations' from a human health perspective in the prevention of communicable diseases. The first pertains to prevention of animal diseases as a strategy to reduce risks of disease on the human side because options for treatment are limited or absent and morbidity and mortality are significant and post exposure prophylaxis (PEP) is costly and of limited availability. Rift Valley fever, rabies and avian and influenza are prime examples. While the majority of Rift Valley infections in humans result in subclinical disease there needs to be raised awareness of the significant mortality and morbidity associated with RVF-associated hepatitis, encephalitis, and haemorrhagic disease and the absence of specific treatment. High risk procedures such as tissue sampling and appropriate personal protection for exposed farm workers need to be reconsidered as evidenced by findings during recent outbreaks in South Africa. Cost effective rabies control and prevention of human cases lies with adequate campaigns and vaccine coverage in dogs with post- exposure prophylaxis regimens being hugely costly, frequently difficult to access and recurrent problems with compliance. The emergence of new influenza subtypes and new zoonoses causing severe respiratory disease has raised many questions around prevention in humans, notably the source of the new corona virus MERS Cov, and influenza A H7N9. The second perspective is prevention of zoonosis in persons at risk because of occupational exposure. These include animal health technicians, veterinarians, zoologists, slaughter men. Cost effective pre- exposure rabies prophylaxis for at risk persons including animal health workers using the intradermal route is considered cost effective but regulatory issues prevent implementation. The third perspective is a comparison of disease prevention programmes in human health versus those in veterinary health and the different challenges. New vaccines and the introduction of some of these vaccines into the Expanded Programme for Immunization have resulted in major decreases in the occurrence of these diseases. Vaccine campaign challenges, politics and the anti- vaccine lobbies affect coverage significantly.

\*Division of Public Health Surveillance and Response, National Institute for Communicable Diseases  
No 1 Modderfontein Road, Sandringham, Johannesburg, South Africa  
lucilleb@nicd.ac.za



## ARE FORMALIN-FIXED-PARAFFIN EMBEDDED TISSUES USEFUL FOR THE DIAGNOSIS OF TUBERCULOSIS IN ANIMALS?

Govender, K.<sup>A,B</sup>, Hlokwé, T.<sup>B</sup>, Michel, A.L.<sup>C</sup>

### SUMMARY

This study was aimed at determining the sensitivity and specificity of the IS6110 specific PCR test on formalin fixed, paraffin embedded (FFPE) tissue blocks, compared to that of the gold standard method culture. A total of 141 FFPE tissue blocks of wild animals from game reserves, the National Zoological Gardens and routine tuberculosis (TB) surveys in Kruger National Park were tested. Among the 50 known TB positive samples the IS6110 PCR had an overall sensitivity of 22%. The positive predictive value of the IS6110 test (91.67%) was very high implying that although sensitivity was low, one can be confident that a positive test result is a true reflection of the positive disease status. The sensitivities of the *M. tuberculosis* and *M. bovis* culture positive samples were compared and a significant difference was noted. Sensitivity of the IS6110 assay in *M. tuberculosis* and *M. bovis* culture positive samples were 66.67% and 8.57%, respectively. Difference in bacterial load in tissues infected with the two mycobacterial species may account for the difference in sensitivities. The limitations with the current protocol probably lie in the low numbers of bacilli in specimens, inefficient DNA extraction of mycobacteria, the presence of PCR inhibitors in the samples and the use of a multisampling protocol, which may have impacted negatively on the test sensitivity. However, the resultant sensitivity was increased when parallel interpretation was applied to histopathology examination and the IS6110 PCR test. Both histopathology examination and PCR tests produce rapid results and their combination can be used in routine diagnostics. Of the 91 known TB negative samples, the specificity of the IS6110 (98.90%) PCR test was high, but the negative predictive value of 69.67%, suggest that the probability of negative test results being incorrect, still exists. Samples diagnosed as “suspect” by histopathological examination and as infected with Mycobacteria other than tuberculosis (MOTT) were classified in the negative sample panel as they contribute to demonstrating specificity of the IS6110 PCR assay. MOTT were cultured from 18 samples which tested negative by IS6110 PCR results (no product amplification) in all cases, indicating that the test is specific enough to prevent misdiagnosis between MOTT and members of the MTB complex. The IS6110 PCR method tested in this study offers a more rapid way of confirming a diagnosis of MTB complex in a sample because it identifies and amplifies the DNA of the organism in question within hours. However, PCR results should always be supported by comparison with conventional diagnostic methods as well as clinical data, if available.

### INTRODUCTION

Bovine tuberculosis is a global cause for concern in livestock, free-ranging wildlife, zoological collections and the human population. A large amount of time, effort and resources are spent on its diagnosis and control methods. In this study the flexibility of this PCR test regarding species variations, organ sample variation, sample size and age of formalin fixed paraffin embedded blocks will be tested. It also lends flexibility to the time period during which samples are fixed in formalin before they can be embedded. The pilot study<sup>9</sup> that was done showed that there is minimal degradation of DNA even for samples that have remained in formalin for up to 3 weeks.

This PCR test would, if successful, hold numerous benefits for both veterinary and human medicine, especially since tuberculosis is a huge problem on a worldwide scale. It could be a valuable diagnostic tool in areas of endemicity, where bovine and human TB coexist and the distinction of *M. bovis* from *M. tuberculosis* is required for monitoring the spread of *M. bovis*.

In the past, differentiation of *M. bovis* from *M. tuberculosis* was based on conventional culture and biochemical tests. In addition to being tedious and slow, these methods were not 100% reliable. Partly due to close genetic relatedness and variable biochemical patterns, definitive detection of *M. bovis* and *M. tuberculosis*, up to species level, was time consuming and difficult. Methods such as PCR, which is currently being used for differentiation, is seen to be the best alternative strategy to meet this purpose<sup>1</sup>.

A Allerton Provincial Veterinary Laboratory, Veterinary Services, Department of Agriculture and Environmental Affairs, Private Bag x2, Cascades, Pietermaritzburg 3201, South Africa  
(Tel: 033 347 6299, email: katt1807@gmail.com)

B Tuberculosis Laboratory, Zoonotic diseases section, ARC-Onderstepoort Veterinary Institute, Private Bag x05, Onderstepoort 0110, South Africa

C Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag x04, Onderstepoort 0110, South Africa

## MATERIALS AND METHODS

### SAMPLES

Formalin fixed, paraffin embedded tissue blocks were selected from the histopathology laboratories at the Faculty of Veterinary Science, University of Pretoria (UP) and the National Zoological Gardens (NZG). A total of 141 tissue blocks that included negative granulomatous lesions, confirmed positive TB lesions as well as suspect TB lesions were collected and taken to the UP Pathology section, where sections with a thickness of 3-4 microns were cut with a microtome and three sections each were placed into sterile microcentrifuge tubes.

Tissue blocks selected were that of organs of animals that could be categorized into 3 groups:

1. Free-ranging wildlife: KNP buffalo (1998 TB survey): all of these samples were cultured at OVI
2. Captive wildlife from the NZG (these samples were either diagnosed at NZG or at the OVI)
3. Incidental TB cases in wildlife (diagnosed by histopathology (UP))

The TB status of these animals was initially determined by either organ culture and/or histopathology of organs.

### DNA EXTRACTION FROM FORMALIN FIXED PARAFFIN EMBEDDED TISSUES

DNA extraction by boiling: Formalin fixed, paraffin embedded sections of approximately 3-4 microns in their respective microcentrifuge tubes were crushed with sterile pipette tips and 200µl of a 0.5% Tween 20(Merck Chemicals) solution added. The tubes were placed in a water bath or heating block and boiled at a 100 °C for ten minutes followed by snap freezing (minus 20 °C freezer) for two minutes. This step was repeated and tubes were boiled for another ten minutes. Without cooling, the tubes were centrifuged for twenty minutes at 3000 x g and the supernatant containing the DNA transferred to new sterile tubes<sup>7,8</sup>.

Table 1: Oligonucleotide primers used for the IS6110 PCR amplification

Primers	IS6110
Forward	CTCGTCCAGCGCCGCTTCGG
Reverse	CCTGCGAGCGTAGGCGTCGG

### PCR PROTOCOL

Ten reactions of a 50µl reaction mix and mastermix for the IS6110 PCR reactions were prepared in a laminar flow cabinet. The IS6110 PCR mastermix was made up of the following: 277.5µl HPLC H<sub>2</sub>O, 50µl 10x Supertherm buffer, 30µl MgCL<sub>2</sub>(25mM), 20 µl dNTP Mix (10mM), 10 µl IS6110 forward primer (20pMol/ul), 10 µl IS6110 reverse primer (20pMol/ µl), 2.5 µl Hot Star Taq (1.25U). Forty microliters of each mastermix was aliquot into pre-labelled 0.2ml thin walled microcentrifuge tubes. Ten microliters of the DNA templates were inoculated into the tubes individually. Finally 10µl of the positive controls (*M. tuberculosis* and *M. bovis*) and the negative control (distilled water) were inoculated into the last three PCR tubes. The PCR tubes were placed in the thermal cycler for PCR amplification.

### DATA ANALYSIS

For the IS6110 PCR, amplification of 123 base pair product for *M. tuberculosis* complex in samples was considered a positive test reaction.

### DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Sensitivity<sup>3</sup> (Se) = Test positives (a) / Known positives (a+c) x 100

Specificity<sup>3</sup> (Sp) = Test negatives (d)/ Known negatives (b+d) x 100  
in a known negative population

### PREDICTIVE VALUES

Predictive value of a Positive test<sup>4</sup> a/(a+ b) = Number of true diseased animals/Total number of positive animals

Predictive value of a negative test<sup>4</sup> d/(c + d) =Number of true non-disease animals/Total number of negative animals

### PARALLEL INTERPRETATION

Histopathology examination and the IS6110 PCR assay were subjected to parallel interpretation<sup>4</sup> to determine whether the sensitivity of their combinations would increase.

## RESULTS

### IS6110 PCR ASSAY

Among the 50 true TB positive samples, the IS6110 PCR identified eleven samples as positive for *M. tuberculosis* complex by amplification of a DNA product with a size of 123 bp. Of the 91 true TB negative samples, the PCR test classified 90 samples as test negative, with one sample showing amplification of a DNA product with a size of 123 bp. The eleven suspect samples were classified as TB negative on this test.

The overall diagnostic sensitivity for the IS6110 assay was 22%. The diagnostic sensitivity among the twelve *M. tuberculosis* and 35 *M. bovis* cultured samples was 66.67% and 8.57%, respectively. The diagnostic specificity for the IS6110 assay was 98.90%.

### PREDICTIVE VALUES

The positive predictive value of the IS6110 PCR was 91.67%. The negative predictive value of the IS6110 PCR was 69.67%.

### PARALLEL INTERPRETATION

Table 2: Comparing sensitivity obtained with parallel interpretation of tests

IS6110 PCR	IS6110 PCR and Histopathology
22%	74%

The calculations in table 2 show that a higher sensitivity can be reached with parallel interpretation of the IS6110 assay with histopathology examination. When both PCR tests are applied to samples the sensitivity achieved is also higher than the overall sensitivity of the application of the individual tests.

## DISCUSSION

With reference to the gold standard diagnostic test method (i.e. culture) for tuberculosis, samples must reach a laboratory within a specific time period. Organs must be transported as aseptically as possible, on ice as soon as possible to a lab for culturing, to prevent autolysis of the sample and destruction of viable organisms. When comparing the above sample collection to the PCR sample collection; formalin-fixed paraffin embedded (FFPE) tissues are easily stored and transported.

Tuberculosis is an important state controlled disease and the successful application of these PCR assays can aid in surveillance, epidemiological study and control of tuberculosis in livestock, wildlife as well as zoo animals. The IS6110 PCR method tested in this study offers a more rapid way of confirming a diagnosis of MTB complex in a sample because it identifies and amplifies the DNA of the organism in question within hours.

In this study we aimed to determine the sensitivity and specificity of the IS6110 specific PCR test compared to that of the gold standard method culture. The results showed that the sensitivity and specificity of the IS6110 PCR assay were 22% and 98.90%, respectively.

The sensitivity (22%) of the IS6110 in this study does not correspond with sensitivities of the IS6110 assay of similar studies<sup>7,9,11</sup>, in which the sensitivities were reported as 98.21%, 39.58% and 93%, respectively, but the positive predictive value of the IS6110 test (91.67%) was very high implying that although the sensitivity was low, one can be confident that a positive test result is a true reflection of the positive disease status. This characteristic can prove very useful in cases where the only sample available is FFPE tissue, such as in an investigation of a valuable zoo animal/collection or retrospective epidemiological studies. The specificity of the IS6110 (98.90%) PCR test was high, but the negative predictive values of 69.67%, suggest that the probability of negative test results being incorrect still exists.

Low sensitivity of the test could be due to the fact that fixatives, including formalin, can markedly reduce the size of DNA fragments that can be recovered, especially if fixation time is prolonged<sup>7</sup>. Another possible reason is that fixation and chemical processing of archival material induces alterations of the DNA and potentially results in complete or partial loss of the DNA<sup>5,11</sup>. False negative results may also be attributed to the presence of DNA inhibitors in the crude tissue extract or the small amount of tissue examined when compared to culture<sup>9</sup>. Studies have also revealed that a multisampling protocol (i.e. sections at different levels of the block) improved the test efficiency because of uneven bacterial distribution<sup>9</sup>. Another possible reason for the low sensitivity could be the DNA recovery or extraction method. The extraction procedure should deliver effective lysis of mycobacteria, good recovery of the DNA from a complex mixture of tissue debris and lastly, removal of PCR inhibitors<sup>7,10</sup>. If the boiling method used in this study could not fulfil these requirements sensitivity is expected to be lower. The DNA extraction method could be changed in order to yield better results.

When the sensitivities for the *M. tuberculosis* and *M. bovis* culture positive samples were compared, a significant difference was noted. Sensitivity of the IS6110 assay in *M. tuberculosis* culture positive samples was 66.67%; sensitivity of the IS6110 assay in *M. bovis* culture positive samples was 8.57%. This could be due to the fact that infection with *M. bovis* has a lower bacterial load in tissues when compared to infection with *M. tuberculosis*<sup>6</sup>. The deposition of connective tissue (encapsulation) is reported to limit the dissemination of *M. bovis* and plays an important role in controlling proliferation of mycobacteria by the entrapment of bacilli inside the lesions<sup>6</sup>.

The specificity of the IS6110 assay 98.90% aligns closely to that of similar studies<sup>7,9</sup>, where the specificities were found to be 100%. The specificity of 98.90% means that only 1 sample in the negative panel showed non-specific amplification of a DNA product with a size of 123bp for the *M. tuberculosis* complex. A possible reason for this false positive result could be contamination of the sample by a TB infected sample during the extraction process or by the positive control during the gel electrophoresis<sup>2</sup>.

Samples diagnosed as “suspect” by histopathological examination and as infected with Mycobacteria other than tuberculosis (MOTT) were classified in the negative sample panel as they contribute to demonstrating specificity of the IS6110 PCR assay. The IS6110 test showed a negative result (no product amplification) for these samples, thus proving that this test is specific enough to differentiate other mycobacteria from members of the MTB complex and identifying true TB negative samples. The specificity of this test is clearly evident from the test results among the negative sample panel. Eighteen samples were cultured MOTT which was paralleled by negative IS6110 PCR results (no product amplification) for all eighteen samples, indicating that the test is specific enough to prevent misdiagnosis between MOTT and members of the MTB complex.

This study shows that the gold standard “culture” is more sensitive when compared to the IS6110 PCR assays, while histopathology can have confounding results (i.e. histopathology examination results suggest lesions associated with TB when culture results confirm TB negative or MOTT). A parallel interpretation of the IS6110 assay and histopathological examination achieved an increased sensitivity of 74% (table 2).

## REFERENCES

1. Bakshi, C.S., Shah, D.H., Verma, R., Singh, R.K. & Malik Meenakshi, 2005, Rapid differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis* based on a 12.7-kb fragment by a single tube multiplex-PCR. *Veterinary Microbiology* 109, 211-216.
2. Borst, A., Box, A. & Fluit, A., 2004, False-positive results and contamination in nucleic acid amplification assays: suggestions for a prevent and destroy strategy. *European Journal of Clinical Microbiology & Infectious Diseases* 4, 289-99.
3. Dohoo, I., Martin, W. & Stryhn, H., 2009, Screening and Diagnostic tests in, *Veterinary epidemiologic research*, 2<sup>nd</sup> edn., Canada: VER Inc., 91-134.
4. Gummow, B., 2005, Applied Epidemiology, *Lecture notes distributed in the unit, PHE 500*, Department of Paraclinical Sciences, University of Pretoria, South Africa in 2005.
5. Lin, J., Kennedy, S.H., Svarovsky, T., Rogers, J., Kemnitz, J.W., Xu, A. & Zondervan, K.T., 2009, High-quality genomic DNA extraction from formalin-fixed and paraffin-embedded samples deparaffinized using mineral oil. *Analytical Biochemistry* 395, 265-267.
6. Menin, A., Fleith, R., Reck, C., Marlow, M., Fernandes, P., Pilati, C. & Ba' Fica, A., 2013, Asymptomatic Cattle Naturally Infected with *Mycobacterium bovis* Present Exacerbated Tissue Pathology and Bacterial Dissemination, *PLoS ONE* 8(1): e53884. doi:10.1371/journal.pone.0053884
7. Miller, J., Jenny, A., Rhyan, J., Saari, D. & Suarez, D., 1997, Detection of *Mycobacterium bovis* in formalin-fixed paraffin-embedded tissues of cattle and elk by PCR amplification of an IS6110 sequence specific for *Mycobacterium tuberculosis* complex organisms. *Journal of Veterinary Diagnostic Investigation* 9, 244-249.
8. Miller, Janice M., Jenny, Allen L. & Payeur, Janet B., 2002, Polymerase chain reaction detection of *Mycobacterium tuberculosis* complex and *Mycobacterium avium* organisms in formalin-fixed tissues from culture-negative ruminants. *Veterinary Microbiology* 87, 15-23.
9. Sethusa, T., 2006, Evaluation of a method used to detect *Mycobacterium bovis* and *Mycobacterium avium subsp. paratuberculosis* in formalin fixed paraffin embedded tissues of domestic and wild animals, MSc thesis, University of Pretoria.
10. Taylor, G.M., Worth, D.R., Palmer, S., Jahans, K. & Hewinson, R.G., 2007, Rapid detection of *Mycobacterium bovis* DNA in cattle lymph nodes with visible lesions using PCR. *BMC Veterinary Research* 3, 1746-6148.
11. Zink, A.R. & Nerlich, A.G., 2004, Molecular strain identification of the *Mycobacterium tuberculosis* complex in archival tissue samples. *Journal of Clinical Pathology* 57, 1185-1192.

# PREVENTIVE ANIMAL HEALTH: HALFWAY INTO THE FIRST DECADE

Magadla, V<sup>\*</sup>, Baloyi, F.M., & Moyo, G.M.

## BACKGROUND

Gauteng is mainly an urban province with little or no attention paid to its rural constituency where 4% of the population is recorded to live. A number of citizens residing in townships and informal settlements practise small scale livestock production, aimed either at the home consumption, income generation or movement of animals to the owner's home provinces to start or restock communal areas. A limited number of farmers located in formal agricultural land are also classified as emerging or small scale or resource poor.

In an endeavour to assist both groups of farmers, the communal /subsistence and small scale, the Primary Animal Health Care programme - a preventive animal health care project, aimed at raising awareness on veterinary and livestock production issues, was launched in 2007.

This presentation highlights major constraints, successes, opportunities and improvements into the project since its inauguration.

The overall objectives:

- Increase the efficiency and effectiveness of animal health care delivery and, consequently, livestock productivity;
- Safeguard public health;
- Support and contribute to the national agricultural development strategy

## MATERIALS AND METHODS

The main activities of the preventive animal health programme are initially based on four pillars. These include; a disease preventative programme which encompasses vaccination campaigns with Supavax (*Bacillus anthracis*, *Clostridium chauvoei* and *Clostridium botulinum*), Lumpy Skin Disease vaccine (Neethling strain) and Pulpvax (*Clostridium perfringens* type D toxoids); treatment and management of external parasite treatments with topical Amitraz/Deltamethrin and internal parasite treatments with albendazoles. The Farmer support pillar is rendered through livestock husbandry skills development and basic clinical care. The last pillar is the introduction of traceability and farmer information system which is on its initial stages. Established stakeholder/inter-sectoral and intergovernmental collaborations have proven a success in assisting farmers with their on-farm infrastructure development, which more often than not is found lacking and therefore a serious hindrance to any activity that requires safe handling of production animals.

## CONCLUSION

We are trying to refine a holistic and sustainable livestock production and health delivery programme that meets the client's/ public's needs in an economical manner and still ensures that government responsibilities of delivery of services, food security, poverty eradication, redress of inequality are fulfilled. *The envisaged end result should be* (1) A public veterinary service that is better able to carry out its responsibilities, as prescribed under the Animal Disease Act, Act 35 of 1984 and Meat Safety Act, Act 40 of 2000, under the new dispensation, i.e. a veterinary service that is both regulatory and developmental in its focus. (2) A functioning private livestock sector; able to support both developing and commercial sectors, rural and urban communities and (3) The necessary resources i.e. knowledge and skills capable supporting extension personnel supplied with the minimum tools and equipment to render value to the farmers, as well as and availability of on-farm infrastructure which enables to the achievement of the overall objective.

## **CONTINUING EDUCATION PRESENTATION: PPR - ONE FOOT IN THE SADC REGION, WHAT OPTIONS EXIST TO STOP IT IN ITS TRACKS?**

Mulumba, M<sup>1</sup>

Abstract not available at time of printing

## EPIDEMIOLOGY OF RECENT FOOT-AND-MOUTH DISEASE OUTBREAK VIRUSES

Blignaut, B.<sup>1\*</sup>, Mlingo, T.<sup>1</sup>, van Heerden, J.<sup>1</sup>, Lukhwareni, A.<sup>1</sup>, Maree, F.F.<sup>1,2</sup> & Heath, L.<sup>1</sup>

### ABSTRACT

Despite the restricted distribution of foot-and-mouth disease (FMD) in the world, it remains a compulsory notifiable disease. The situation in Africa is complicated by genetic and antigenic variability of the South African Territories (SAT) types. Although SAT2 type virus is the causative agent in most outbreaks in southern Africa, the SAT1 types are widely dispersed, whereas SAT3 type viruses are the least associated with outbreaks in domestic animals. Several outbreaks have occurred in South Africa since the turn of the century, e.g. SAT1 outbreaks were reported during 2000/2002-2003/2009-2011, SAT2 outbreaks during 2001/2003-2005/2009/2011-2012 and a SAT3 outbreak in 2006 emphasising the need for ongoing disease surveillance.

FMD molecular epidemiological studies for the SAT type viruses have mainly concentrated on the 1D-coding region to determine genetic relatedness of isolates. Data on the external capsid-coding region (P1) is inadequate due to the restricted numbers and the fact that the majority are historic isolates that do not shed much light on the current epidemiological situation. Characterisations of the P1 region provide important data on current circulating SAT type viruses, and possible epitopes that may be important for inclusion into FMD vaccines. Therefore the genetic variability of the SAT types in southern Africa was investigated to evaluate the characteristics of recent field isolates from wildlife, as well as livestock. The viruses were chosen to be representative of what is currently in the field and to cover the three topotypes found within southern Africa (South Africa, Namibia, Zimbabwe, Botswana, Zambia and Malawi). The viral RNA was amplified by reverse transcriptase-PCR, purified and used for sequencing. The nucleotide and deduced amino acid data was analysed for genetic and phylogenetic comparisons. Furthermore, multiple isolates of the SAT1 type outbreaks in South Africa (2009 and 2010) were isolated and characterised. Phylogenetic analysis of recent outbreak viruses revealed their genetic relatedness to other South African isolates, as well as FMDV from the Kruger National Park which grouped according to geographical origin (topotype 1). Greater genetic heterogeneity was observed for SAT1, SAT2 and SAT3 compared to other serotypes. Comparison of the SAT1 and SAT2 viruses revealed 51% variable nucleotides for the capsid-coding region. The amino acid variation of the P1 sequences between the SAT1 and SAT2 type viruses was 36% and 38%, respectively. Both the nucleotide and amino acid variation of the P1 were least for the SAT3 viruses. Hypervariable amino acids on the capsid protein were identified and coincided with previously identified epitopes. The SAT1 and SAT2 types were more variable compared to SAT3 for which the least hypervariable regions were observed. This emphasises the need for ongoing epidemiological studies investigating the P1 region of FMDV, contributing to knowledge about the disease and improved control in southern Africa.

<sup>1</sup> Transboundary Animal Diseases Programme, Onderstepoort Veterinary Institute, Agricultural Research Council, Onderstepoort, 0110, South Africa.

<sup>2</sup> Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa.

\*Contact author:

Telephone +27 12 529 9594/84

Email bohmerb@arc.agric.za



## INDIRECT ASSESSMENT OF VACCINE MATCHING FOR SOUTHERN AFRICAN FOOT-AND-MOUTH DISEASE VIRUSES

Blignaut, B.<sup>1\*</sup>, Lukhwareni, A.<sup>1</sup>, Mlingo, T.<sup>1</sup>, Esterhuysen, J.J.<sup>1</sup>, Fosgate, G.T.<sup>2</sup> & Maree, F.F.<sup>1,3</sup>

### ABSTRACT

Foot-and-mouth disease (FMD) vaccine candidates should be closely related to emerging strains and induce immune responses with broad immunological cross-reactivity for appropriate protection against FMD viruses (FMDV) from different geographic regions (topotypes). Since 2000, several outbreaks of FMD have occurred in southern Africa emphasising the need for quick and cost-effective methods to assess the suitability of vaccine strains. Cross-protection of FMD vaccines are assessed by *in vivo* potency tests. However, animal trials are costly and laborious and indirect vaccine matching tests such as serological  $r_1$ -value determination are practical alternatives. The  $r_1$ -value is determined according to the ratio of the reciprocal serum titre to the heterologous virus against the reciprocal serum titre to the homologous virus. Antigenic relationships were established examining 26 recent SAT1 outbreak viruses tested against reference sera representing existing vaccine strains and circulating field isolates from southern Africa (topotypes 1, 2 and 3). Convalescent bovine sera (5), as well as pooled serum samples were analysed by the virus neutralisation test (VNT). Statistical analyses of neutralising antibody data were employed to determine factors that contribute to variability in the VNT and to investigate antigenic relationships of these viruses with reference strains. Two models based on the test virus and test virus topotype were fit independently and topotype classification accounted for approximately 50% of the variability within test viruses. The variability attributed to components of the VNT was highest for the test virus (23.9%) and topotype (11.0%) compared to the reference sera (*ca.* 3.8%), operator (*ca.* 1.4%) and animal (4.2%). Mean titres obtained from individuals were strongly correlated with pooled sera ( $r = 0.826$ ;  $P < 0.001$ ) and were not significantly different ( $P = 0.797$ ). The advantage of using pooled sera is a shorter turn-around time and more cost-effective diagnostic testing. A good vaccine match ( $r_1$ -values ranging between 0.2-0.4 and  $>0.4$ ) were observed for most viruses tested against topotype 1 and 2 reference sera. Furthermore, varying levels of cross-neutralisation were observed specifically for viruses tested against the topotype 3 reference sera. This suggests that topotype 3 viruses are antigenically more disparate and that a vaccine consisting of topotype 1 or 2 antigens may not be effective in the control of FMD. These results emphasise the need for antigenic variability studies on SAT type viruses in conjunction with improved vaccinology.

<sup>1</sup>Transboundary Animal Diseases Programme, Onderstepoort Veterinary Institute, Agricultural Research Council, Onderstepoort, 0110, South Africa.

<sup>2</sup>Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa.

<sup>3</sup>Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa.

\*Contact author:

Telephone +27 12 529 9594/84

Email bohmerb@arc.agric.za

# THE RISK OF DISEASE SPREAD THROUGH WILDLIFE TRANSLOCATIONS

De Klerk-Lorist, L.\*

## ABSTRACT

Wildlife disease surveillance, investigations, diagnostics and management are some of the key aspects of conservation medicine. Veterinarians are well trained in recognizing, identifying, diagnosing and treating disease in individual domestic animals or captive wildlife, but in free or semi free-ranging wildlife populations disease eco-epidemiology at the population level is more difficult to investigate and is of greater importance. Any veterinarian involved in wildlife disease investigation and conservation medicine therefore needs to have a good background in the basic principles of wildlife biology, species behavioural ecology and disease epidemiology.

Previously in 2013 the Contingency protocol for dealing with buffalo and buffalo movements was circulated to all provincial directors. It was also stated that climate change and movement of animals have led to the spread of brown ear tick occurrence in South Africa. Animal movements, with specific reference to movements and uncontrolled translocations of wildlife might be playing an increasingly bigger role in certain disease epidemics in semi free-range wildlife populations as well as domestic stock. It is speculated that there might be many more diseases carried by wildlife, but unfortunately this is becoming increasingly difficult to prove due to lack of official control or monitoring strategies for wildlife as well as the absence of validated disease screening tests in these species.

\*Department of Agriculture, Forestry and Fisheries  
LinmarieDK@daff.gov.za

# **HOLISTIC PET CARE PROGRAM – “GIVE YOUR PET A NEW LEASH IN LIFE”: A REVIEW OF PROGRAM IMPLEMENTATION (2008 – 2013)**

Magadla, V\*, Moyo, G.M. & Baloyi, F.M.

## **BACKGROUND**

While Gauteng Province is the home of the veterinary science faculty and hosts the greatest percentage of Private Veterinarians in the country, access to veterinary assistance for the majority of pet owners, particularly those living in informal settlements and low income areas, is minimal or absent. The reasons for this could be either a lack of access (no or very few private veterinarians would even consider opening a clinic in townships and informal settlements, for both founded and unfounded reasons) and/or a perceived lack of affordability amongst the pet owners.

As a means of working towards closing this gap, the holistic pet care programme was launched in 2007. The main objective of the programme is to create awareness on pet care through rendering basic pet care services to the indigenous communities, which are mainly marginalized. The programme is working towards bridging the gap on access to veterinary services and it ultimately aims at the enhancement of responsible pet ownership resulting in improved pet health which positively impacts on public health and social wellbeing.

This is the review of the progress made within the programme over the past five years

## **MATERIALS AND METHODS**

The launch of the programme in 2007 was based on the slogan, “Give your pet a new leash in life”, directed towards promoting responsible pet care and ownership in our communities. The first phase, which was a pilot project which aimed at least 3500 pets, entailed a planned vaccination campaign ahead of the sterilization campaign, in the 20 priority townships. The canines received a 6-in-1 vaccine (attenuated canine distemper virus, canine adenovirus type 2, parvovirus, canine parainfluenza virus with inactivated leptospira and rabies suspension as diluent) whilst the felines were given a 4-in-1 vaccine (Feline calici virus infection, rhinotracheitis, panleukopaemia and rabies viruses). Public veterinary awareness and extension is offered through schools and departmental stakeholder engagement/capacity building workshops. Annual international animal health awareness campaigns e.g. World Rabies Day, in partnership with other government departments; private sector and academic institutions, continue to be a priority. With limited resources at hand, and in our endeavour to establish sustainable pet care, we continue to support limited clinical services rendered by Community Veterinary Clinics – A SAVA social responsibility program. This public-private partnership ensures a win-win-win situation for all stakeholders; government facilitates the provision of a critical and needed service, SAVA and its members achieve a key strategic imperative and of course pet owners get to benefit from this facilitated pet health service.

## **CONCLUSION**

We strongly believe that the future of veterinary public health and public health generally, is limited only by the ability of the veterinary and para-veterinary professions to recognize its opportunities and accept its responsibilities. We are committed to complementing and enhancing the competitor’s goals and objectives and letting the best and the great idea win. It continues to be our responsibility and we never stop trying to expand the circle of the veterinary and para-veterinary professions in our sphere of influence. As is always the case, going forward there is a need to quantify the real impact that this service is having in the welfare of the pets and in the overall improvement of the social health and wellbeing of the pet owners.



---

# Proceedings of the 11th annual congress of the Southern African Society for Veterinary Epidemiology and Preventive Medicine

---

