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CONTINUING EDUCATION PRESENTERS

Prof. Lucille Blumberg

Deputy-Director: National Institute for Communicable Diseases, National Health Laboratory Services

Prof. Alan Guthrie

Equine Research Centre, University of Pretoria

Dr. Frederick Kivaria

National Epidemiologist, Tanzanian Ministry of Livestock Development and Fisheries

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THE CONTROL OF MEASLES [BOVINE CYSTICERCOSIS (*TAENIA SAGINATA*)] FROM SOUTH AFRICAN FEEDLOT CATTLE: WHERE DO WE COME FROM, WHERE ARE WE NOW AND WHERE ARE WE GOING WITH CONTROL & PREVENTION PROGRAMMES AGAINST THIS ZOONOSIS?

Verwoerd,D.J¹ & Prozesky,L²

SUMMARY:

Post mortem meat inspection of so-called predilection sites have been the mainstay of control measures against this helminthic zoonosis by Veterinary Public Health authorities worldwide for more than 30 yrs. Recent studies and technological advances in diagnostics have demonstrated the inadequacies of this approach, especially regarding cattle, while the enforcement of current decontamination procedures such as freezing carcasses at -20 °C for 10 days causes massive financial losses by the South African beef industry, with no demonstrable benefits to consumer health. A retained measles carcass loses approximately 20% of its monetary value. This paper reviews abattoir data from 1 022 556 cattle slaughtered during 2009-2011 at the largest export approved facility in the country. A total of 24443 carcasses were diagnosed with measles. The prevalence of measles was on average 2,4% per month with a maximum of 4,8% and a minimum of 1%. Accurate electronic origin and production data was available for 22770 measles cattle (93% match) allowing more detailed analyses. The epidemic nature of bovine cysticercosis in this dataset is discussed against the background of current epidemiological knowledge regarding *Taenia* spp. in general and cattle feedlots in particular. Measures to improve the specificity of measles diagnosis during meat inspection included monthly histological examination of suspected lesions on selected days. Incorrect diagnoses dropped from > 20% to <5%. Data from 3805 histological samples indicate that 9% to 42% (avg =23%) of detected measles cases during this period represent recent infection, probably within the feeding period. Old, calcified, non-infectious lesions represented 22% to 63% (avg =45%) of lesions. More sensible control measures instead of the current outdated and wasteful approach are discussed and a thorough revision of State regulatory control recommended.

INTRODUCTION: WHERE DO WE COME FROM?

Cysticercosis and Taeniosis refer to cosmopolitan foodborne zoonotic infections associated with tapeworms where the former indicate a tissue infection caused by the larval cysticercus or metacestode stage, most commonly in pork (*Taenia solium*) and pig organs (*Taenia asiatica*) & cattle (*Taenia saginata*). The adult stage develops only in the intestine of the human (obligate) host, and is acquired through eating of improperly cooked infected meat. **Uniquely** the larval/cysticercus stage of *Taenia solium* can also infect humans and cause cysticercosis/neurocysticercosis, **this is NOT the case with the cattle tapeworm *Taenia saginata***. (Murrell 2005). This and other differences are summarised in Table.1 and have often been overlooked during efforts to control the public health impact of these zoonoses. Neurocysticercosis is now accepted as one of the most important causes of epilepsy, particularly in developing nations characterised by poor sanitation, dependence on contaminated surface water, rapid increase in smallholder pig production and close contact between humans and pigs, and is now the subject of a global public health campaign (“Out of the Shadows”). Human cysticercosis control in Eastern & Southern Africa is coordinated by a dedicated Working Group of Medical-, Veterinary-, and Social scientists established in 2002. (Anon.2007). The relatively simple lifecycle indicate that any interruption of the link between intermediate (pigs, cattle) and definitive hosts (humans) will lower the risk of contracting taeniosis /cysticercosis, so a major effort have traditionally been the emphasis on the identification and treatment of infected carcasses at slaughter, to prevent their assimilation in the human food chain. However these zoonoses persist, especially in areas where poor sanitation, ineffective sewage treatment plants, irrigation with raw sewage/heavily contaminated surface water, free range or smallholder pig production and informal slaughter practices prevail. This is due to the enormous reproductive potential of Taenaeiid tapeworms where adults measuring 1-12m release one or more gravid proglottids (segments 0.5cm x 1-2cm sized) daily, each containing 50 000 to 80 000 eggs.(Schmidt 1986, Hyman 1951).

1. Dirk J.Verwoerd

Staff Feedlot Veterinarian: Karan Beef:

P.O.Box 35

Heidelberg, Gauteng 1438

Tel.016-3421214

Fax.016-3421212

dirkv@karanbeef.com

2. Dept Paraclinical Sciences,

Faculty of Veterinary Science, Onderstepoort

MEAT INSPECTION:

Post mortem meat inspection and sanitizing of infected carcasses in most countries are standardised according to codes of practice summarised by the Joint FAO/WHO Food Standards Programme and Meat Hygiene textbooks (Gracey 1999). Freezing of infected carcasses at -20 °C for 10 days is the recommended practice, but the associated additional costs devalue the carcasses with 20%, causing massive financial losses to the SA beef industry. No distinction is made in the relative contribution to the zoonotic public health risk of cysticercosis between pig tapeworm (*Taenia solium*) and cattle tapeworm (*Taenia saginata*) despite the important epidemiological differences between the two. This is reflected in the vast amount of scientific literature describing the association between pig tapeworm (*Taenia solium*) and human cysticercosis/neurocysticercosis/epilepsy while direct evidence of cattle tapeworm (*Taenia saginata*) and these human conditions are absent and largely unsubstantiated extensions of data from *T. solium* resulting in generic “*Taenia* control measures” with regulations written in the 1970’s based on the precautionary principle. (Murrell 2005).

Even standardised meat inspection practices are very subjective and notoriously inconsistent with both the specificity and sensitivity dependant on the experience of the Meat Inspector.(see Discussion in this paper).This ability varies widely between abattoirs in South Africa. Most low volume red meat abattoirs in South Africa do not have fulltime State Meat Inspectors or even have adequate freezing facilities to sanitize infected carcasses. It is an open secret in the industry that the official cysticercosis prevalence in these establishments approach 0. Meat inspection procedures are more efficient in detecting heavy infections than light /moderate infections.(Kyvsgaard, Ilsoe, Henriksen & Nansen 1990).The very low sensitivity of routine meat inspection to detect *T.saginata* cysts is illustrated by a study of 79 calves aged 2-12m from a known *T.saginata* endemic area in Europe. At dissection 75.9% were found infected but only 38.3% were detected at meat inspection. Of these 21.7% had cysts in the triceps muscle only. This study as well as several others also questions the value of a reliance on so-called predilection sites (masticatory muscles, tongue, heart, oesophagus and diaphragm) as 34/60 infected cattle (56.7%) were negative for cysts in these areas. (Walther & Koske 1980, Murrell 2005). Although these sites had a higher density of cysts the available evidence indicate that there are no favoured sites which if found uninfected would guarantee freedom of the carcass from cysticerci.(Murrell 2005). It should be clear that meat inspection is an outdated, unreliable and ineffective tool to prevent human taeniosis and in the case of *Taenia saginata* that do not cause human cysticercosis at all, completely inappropriate and a total waste of both financial and human resources. At the moment it is only a penalty totalling millions of rands to the higher profile beef producers, with absolutely no impact on human cysticercosis.

Table 1. Differences between pig tapeworm (*Taenia solium*) and cattle tapeworm (*Taenia saginata*) that are important for effective control measures.

Pig Tapeworm (<i>Taenia solium</i>)	Cattle Tapeworm (<i>Taenia saginata</i>)
Larval stage can infect not only pigs but also humans to cause cysticercosis.	Larval stage can only infect cattle to cause cysticercosis.
Gravid proglottids containing eggs are shed passively in the faeces.	Gravid proglottids containing eggs are disseminated by actively crawling out of the anus as well as passively shed in the faeces.
Overwhelming evidence indicate that <i>Taenia solium</i> is the cause of Human cysticercosis/ neurocysticercosis and related epilepsy. Conditions often difficult /dangerous to treat.	Very little if any direct evidence link <i>Taenia saginata</i> with human disease other than relatively innocuous taeniasis (adult tapeworm in the intestinal tract), a condition easily treated with standard antihelmintics.

IMMUNODIAGNOSIS:

Sero-epidemiological studies [Ag & Ab-ELISA] reveal a similar pattern where 2x to 3x more infected carcasses were detected compared to routine meat inspection in Kenya (Onyango-Abuje et al 1996).Similarly, in a Belgian study using 1164 sera from 20 export abattoirs, Ag-ELISA detected 36 (3.09%) positives while meat inspection detected only 3 (0.26%), a 12x difference.(Dorny 2000). High specificity is associated with MAb based IgM- ELISA (93.4%) and IgG-ELISA (98.7%) as well as a sensitivity of 92% with cattle carrying >50 cysts. However the sensitivity dropped significantly when cattle carried <50 cysts and only a very small % were detected.(Van Kerckhoven et al 1998).An experimental study in Denmark indicated that cattle which harboured less than 41 viable cysts did not react to detectable levels with a MAb-based IgG ELISA .(Bogh et al 1996). Antibodies were detected by 3 weeks post infection (p.i) in experimentally infected calves and parasite antigen by 4-7 weeks p.i, but only in calves with 14+ live cysticerci. In naturally infected cattle only 22% (5/23) with 1-29 live cysticerci were detected with Ag-ELISA. Such low sensitivities have precluded the development of practical immunodiagnostic ante mortem tests in cattle.

OUTBREAKS IN FEEDLOT CATTLE:

Cysticercosis follows epidemic outbreak patterns in a cattle feedlot. (see Fig .1) These peaks / point epidemics have been related to contaminated feed raw materials (e.g. potato by-products) (Yoder 1994), feed and/or water contamination by specific infected workers (Slonka 1978, Murrell 2005) as well as sewage irrigated pastures (Fertig 1985, Rickard 1977). In practical terms in South Africa the latter also occurs when cattle are grazed in vleis & floodplains surrounding informal settlements / municipal areas or any area inundated with overflow of raw sewage from overwhelmed / non-functional sewage treatment plants. According to the Green Drop Report of July 2011 from the office of the Minister of Water & Environmental Affairs less than half of SA's 821 municipal sewage works are functional. Thus sewage contaminated water both directly as well as indirectly constitute a growing risk factor associated with cysticercosis in cattle.

MATERIALS AND METHODS

Retrospective analysis of all abattoir data from 1 022 556 cattle slaughtered during 2009-2011 at the largest volume export approved facility [Karan Beef Balfour Abattoir] in South Africa was done for this study. Only data from feedlot cattle transported from the company's own feedlot in Heidelberg with a standing capacity of 120 000 head were used. Positive cysticercosis (= "measles") carcasses were related to their feedlot production histories as well as source and geographical area of origin. Previous spurious histological investigations at this abattoir have indicated a large % of false positive diagnoses during routine meat inspection. During 2011 all "measles" lesions were sampled and placed in 10% buffered formalin in individual containers marked with the sequential slaughter number of the carcass, allowing traceability of the entire history of the specific animal, and routinely processed and stained with haematoxylin and eosin (Anon, 1968). Two days each month were randomly selected (one "high" and one "low" number) and sent for histological examination by a single very experienced veterinary pathologist [L Prozesky] . Lesions were histologically classified as: **Viable/Degenerative** suggesting recent infection probably during the feeding period; **Necrotic** suggesting a successful, completed immune response; **Mineralised/Calcified** suggesting old infection probably before the entrance into the feedlot; **Granulomatous reaction** suggesting an active, ongoing immune response; **Eosinophilic reaction** suggesting an active inflammatory/hypersensitivity response; and **False/ No Cysticercus** suggesting false meat inspection diagnosis or incorrect sampling technique.

RESULTS & DISCUSSION: *WHERE ARE WE NOW?*

The prevalence of cysticercosis as determined by routine meat inspection over the entire 36 months were 24 443 carcasses = an average of 2.4% with a maximum of 4.8% and a minimum of 1%, displaying an irregular epidemic outbreak pattern. See Fig 1. Average infection rate was 2.4 per lot of on average 90 head of cattle, however the distribution of infection between lots was dramatically skewed: the 10% worst infected lots contributed 24% of all cysticercosis carcasses, while 50% of all cysticercosis carcasses were from only 28% of lots received during this period. Consistent patterns were identifiable in terms of buyer, source (cow-calf herd, speculator, auction), origin and loading point and further similar purchases stopped. (data not shown). No significant patterns regarding sex or incoming weight category were detected, while several of the worst infected lots also had a significantly higher than expected respiratory disease morbidity & mortality rate. This positive correlation is unlikely to be causative, but would rather indicate a common calthood history characterised by poor nutrition, poor colostrum transfer, several owners etc. typical of an auction/speculator source. This profile further strengthened the recommendation to rather not buy such lots at all.

The limitations of meat inspection diagnosis described above could significantly influence the accuracy of these results in terms of the true prevalence. Analysis of seasonal patterns from areas of origin were not attempted but could well be relevant because of the influence of rainfall & flooding on pasture contamination..

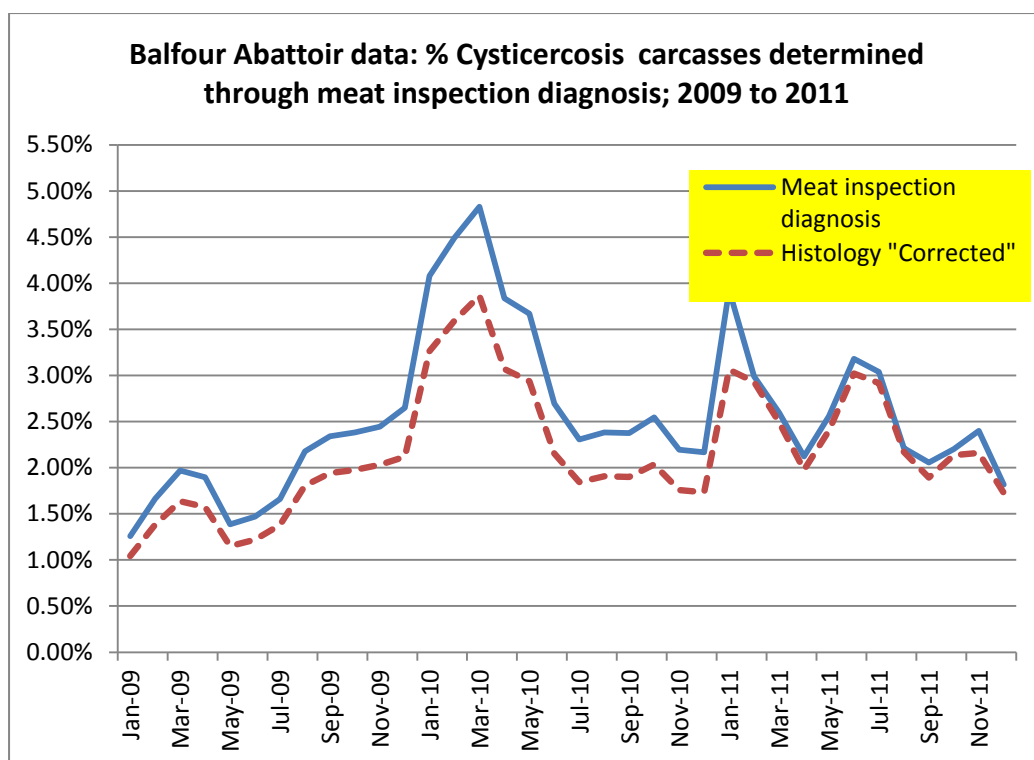


Figure 1. Monthly % Cysticercosis cases as determined through routine meat inspection at Balfour Abattoir 2009-2011. Dotted line indicates the theoretical 'corrected' figure based on the % false/no diagnoses as determined by histology. Note the improvement since Feb 2011.

Three thousand eight hundred and five (3805) samples were examined histologically (Table 2.) suggesting a significant 15-26% infection contribution as a result of an intra-feedlot cycle, while 35-47% were most probably bought in already infected. Even though the larvae require at least 12 weeks to develop to fully infective cysticerci, (Murrell 2005) the characteristic bladder form may be identified in muscle within 2-4 weeks after infection. This classification remains subjective thus the % between these extremes remains as an indeterminate category. Historically approximately 20% of diagnoses were false positives / incorrect sampling, this improved to an average of 5% after instituting a programme whereby suspected measles were retained for a second opinion by designated experienced independent meat inspectors / State Veterinarian [Dr D Lloyd] .

Table 2. Histological categories of suspected measles samples:

Month Slaughtered	Viable feedlot cycle	Unknown time estimate	Mineralization; Purchased	Incorrect Diagnosis
Jan-11	15%	5%	59%	22%
Feb-11	21%	35%	42%	2%
Mar-11	22%	27%	47%	4%
Apr-11	25%	15%	53%	7%
May-11	20%	42%	32%	6%
Jun-11	26%	36%	34%	4%
Jul-11	21%	43%	31%	5%
Aug-11	34%	1%	63%	2%
Sep-11	24%	21%	47%	8%
Oct-11	42%	22%	32%	3%
Nov-11	9%	30%	51%	10%
Dec-11	19%	53%	22%	5%

The analysis of this slaughter and histology data stimulated the gradual introduction of Impact Mitigation Strategies on the following 3 focus areas, with all 3 emphasised during 2011:

Table 3. Focus strategies to mitigate the impact of bovine cysticercosis:

Infected purchased cattle (old infections)	Identification of epidemiological footprint of “10% worst infected Lots”; stop purchasing them.
Feedlot infection cycle (recent infections)	Upgrading and emphasis of obligatory deworming of all feedlot staff every 3 months as well as temporary labour.
False diagnoses at Abattoir	“suspected measles” 2 nd opinion verification programme

Indications are that these actions do indeed make a positive difference in mitigating the financial impact of Bovine Cysticercosis control measures, but current scientific knowledge forces us to seriously re-evaluate the justification of current bovine cysticercosis control measures at abattoirs.

FUTURE CONSIDERATIONS: WHERE ARE WE GOING?

There should be a clear distinction made between pig associated tapeworms (*Taenia solium* & *Taenia asiatica*) causing taeniosis AND cysticercosis in humans and **bovine tapeworm (*Taenia saginata*) causing only taeniosis** in humans. Limited financial and human resources in the State Veterinary structure should be redeployed to address the situation in an epidemiologically correct manner with **total emphasis on pigs**, especially smallholder & free range pigs. Simple deworming programmes in affected communities / high risk employees plus education drives have the biggest impact on human cysticercosis prevalence.(Murrell 2005)

Further continuation of ineffective, inappropriate and wasteful meat inspection practices at beef abattoirs should be **stopped** in terms of State regulatory control, with the responsibility of prevention of beef cysticercosis transferred to the Beef Industry and the responsibility of prevention of human taeniosis transferred to the consumer.[“Wash your Hands and Cook your Food”].

Recently developed recombinant vaccines showing extraordinary efficacy in intermediate hosts (pigs, cattle, sheep) against both Cestode tapeworms (*Taenia* spp) as well as Echinococcus tapeworms (Hydatid cysts) should be aggressively pursued and supported so these products can become available commercially as soon as possible. They will play an enormous role in conjunction with strategic deworming to prevent human cysticercosis in future. Increased understanding of the concept of “concomitant immunity” which forms the immunological basis for the efficacy of these vaccines, also allow improved predictive interpretation of cysticercosis cysts found during abattoir inspection; e.g. the cysticercosis carcasses with calcified cysts (average of 45%; Table.2) found in this study indicate that all other (not seen) cysts in these carcasses are also calcified and thus non-infective.(Lightowlers 2010, Lightowlers, Colebrook Gauci, Gauci, Kyngdon, Monkhouse, Vallejo Rodriguez, Read, Rolfe, Sato 2003).

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EXTENDED ABSTRACT:

THE FULL PAPER IS PRESENTLY UNDER CONSIDERATION FOR PUBLICATION IN THE JOURNAL, PREVENTIVE VETERINARY MEDICINE

EXPOSURE FACTORS ASSOCIATED WITH FMD VIRUS INFECTIONS IN CATTLE HERDS IN NIGERIA DURING 2007-2009

Fasina^a, F. O., Connell^b, D. R., Talabi^c, O. A., Lazarus^a, D. D. & Hernandez^b, J. A.

SUMMARY

New outbreaks of foot-and-mouth disease (FMD) were recorded in cattle herds in Nigeria during 2007-2009. In this study, we aimed at identifying the exposure factors associated with infection and serodiagnosis of FMD in cattle herds. Cattle herds in an FMD affected neighbourhood had higher odds of being seropositive to FMD, compared to herds that were in a neighbourhood not affected with FMD (OR = 16.27; 95% CI = 3.61, 18.74; $P < 0.01$). Cattle herds that share water points along the trek routes with other cattle herds also had higher odds of being seropositive to FMD (adjusted OR = 4.15; 95% CI = 0.92, 18.74; $P < 0.06$). Results from this study can be used by veterinary services in Nigeria and other countries to improve control and eradication programmes for FMD.

INTRODUCTION

Foot and mouth disease virus (FMDV) is an RNA virus of the *Picornaviridae* family that naturally infects cattle and other livestock species, causing an acute illness characterized by lameness and vesicular lesions in the buccal cavity, interdigital space and teats. FMD rapidly infect cloven-footed animals, and is endemic in Sub-Saharan Africa causing major economic losses in the livestock industry. Six serotypes (except Asia-1) have been identified in Africa and four in West Africa (A, O, SAT-1, and SAT-2). In recent times (2007-2009), Serotypes O, A and SAT-2 circulated and were isolated in Nigeria with genetic links to those that caused outbreaks in other parts of West and Central Africa (WRLFMD, 2012). However, data on the risk factors associated with these outbreaks were lacking. We evaluated exposure factors for FMD in the Nigerian cattle and suggest control strategies.

MATERIALS AND METHODS

States of Adamawa, Bauchi, Niger, Plateau and Ogun in Nigeria were selected for the study in view of their importance in cattle movements and trades in Nigeria (see figure). The country has a cattle population of ≈ 16 million (FAO, 2012) and many cattle heads move in or out of the country daily since the country serves as a major meeting point for most of cattle arriving from The study populations include subsets of sedentary herds, pastoralist herds, cattle markets, and other cattle herds.

Sero-epidemiological study using FMD NSP 3-ABC ELISA (Prionics, Lelystad B. V., the Netherlands) was done to identify all sero-positive and sero-negative farms and a total of 68 case and control farms each (total=136) was included for the case-control study. Samples (tissues and sera) from clinically sick or apparently healthy animals were also submitted to the FAO World Reference Laboratory for Foot-and-Mouth Disease for confirmation of diagnosis carried out at the National Veterinary Research Institute, Vom, Nigeria. An animal sero-positive for the ELISA test was taken as exposed to and infected with FMD since vaccination is rarely carried out in Nigeria or done sparingly using NSP-free vaccine which produces antibodies against SP, but not against NSP (Brocchi et al., 2006; Engel et al., 2008). Samples with doubtful results were retested in pairs to confirm FMD statuses of animals.

Pre-tested and validated structured questionnaire was used to collect herd-level exposure factors' data. The questionnaire was prepared in three dialects (i.e., Fulfulde, Hausa and Yoruba) for easy communication with producers in different ethnic groups. Twenty-nine factors including those related to origins of animal, management, biosecurity and movements were tested using univariable conditional logistic regression model to associate the odds of being a case herd as a function of each investigated exposure factor. Exposure variables with P values ≤ 0.20 [using X^2 test, (two tailed), and biological plausibility) were retained and considered for inclusion in a multivariable logistic regression analysis. Confounding was tested for and a final visual examination of residual plots following model fits (standardized delta-beta values vs. observation number and delta-beta values vs. fitted values) were done. The adjusted OR and 95% confidence interval (CI) were reported.

a Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private bag X04, Onderstepoort 0110, Tel: +12 529 8069; South Africa.

b Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

c College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria.

* Contact details of corresponding author: Department of Production Animal Studies, University of Pretoria, Onderstepoort 0110, South Africa. Telephone: +27 12 529 8069; Fax: +27 12 529 8396; Email: dayo.fasina@up.ac.za

RESULTS

This present study identified 68 case herds and 68 control herds in Nigeria. Nine virus isolates were recovered at the WRLFMD from the case herds. Most case and control herds were herds with ≤ 50 cattle head (data not shown). In the univariable analysis, 14 variables had values of $P \leq 0.20$ and were further analysed for biological plausibility, magnitude of association, and statistical significance. The explanatory variable for 'FMD outbreak in the neighbourhood' was associated ($P < 0.05$) with the variable for 'cattle market in the neighbourhood'. The variable for 'farmer and cattle herd share water point along the trek routes with other herds' was associated with the variables for 'neighbouring village share water points' and 'neighbouring village share grazing reserves'. The variable 'neighbouring village share water points' was associated with the variable for 'neighbouring village share grazing reserves'. Finally, the variable for 'neighbouring village share grazing reserves' was associated with the variable for 'farmers and animals share trek route'.

In the multivariable analysis, the variables for 'FMD outbreak in the neighbourhood' and for 'farmer and cattle herd share water point along the trek routes with other herds' were retained in the final model. Addition to the model of the interaction term between these two variables was not significant ($P = 0.48$) and this term was removed from the model. Visual examination of residuals revealed that delta-beta values for the two variables kept in the final model were not extreme (i.e., not > 1), which supported overall goodness of fit. Analysis of residuals (set of case and control herds with the largest delta-beta value and lowest fitted value) indicated the existence of influential observations; and the removal of these observations did not change the finding of greater risk associated with 'FMD outbreak in the neighbourhood' and for 'farmer and cattle herd share water point along the trek routes with other herds'.

DISCUSSION

This study produced epidemiologic evidence that both direct and indirect transmission of FMD occurred between cattle herds caused by a neighbourhood effect and by cattle herds sharing water points. Cattle herds in a neighbourhood affected with FMD had higher odds of being classified as seropositive to FMD, compared to herds that were in a neighbourhood not affected with FMD (adjusted OR = 16.27; 95% CI = 3.61, 18.74; $P < 0.01$). Neighbourhoods in rural Nigeria allow for direct and indirect contact between animals. Cattle herds are not confined in fenced premises. Usually, cattle (particularly calves) are not penned/tied down at night, and this practice can be a source of direct and indirect contact between cattle. A neighbourhood effect for FMDV transmission between herds has been identified in other countries. Airborne spread of FMD from swine to nearby cattle and sheep, and an increase in the movements of vehicles and personnel engaged in disease control efforts perhaps facilitated local spread of FMD during the epidemic in the UK in 2001 (Mansley et al., 2011; Ellis-Iversen et al., 2011).

In this study, cattle herds that share water points along the trek routes with other cattle herds had higher odds of being classified as seropositive to FMD (adjusted OR = 4.15; 95% CI = 0.92, 18.74; $P < 0.06$). In Nigeria, there are established old grazing reserves and watering points along the trek routes, and animals originating from different locations share these reserves and watering points. In addition, rivers crossing the routes of these animals are shared along the routes and, oftentimes, both FMD-infected and susceptible cattle herds are moved together with shared facilities. A similar type of exposure and disease transmission between FMD-infected and susceptible animals has been documented in other studies in South Africa. For example, at the Kruger National Park, between the months of May and November, when the water is scarce in the Park, buffalo infected or potentially infected with FMDV congregate around available water points providing an opportunity for direct and indirect contact with susceptible domestic livestock (Jori et al., 2009).

The limitations of this study include: (1). FMDV isolates were not recovered all of the samples submitted per case herd. It is possible that improper sample collection and processing (or packing) combined with a long distance and time required for samples to reach the NVRI in Vom could have affected the quality (freshness) of the sample to recover FMDV. (2). Second, since certain herds had only one sero-positive animal, and these herds were not confirmed as infected by virus isolation or rt-PCR, there is a possibility of misclassification of one or more case herds as the ELISA test may not be perfect (Brocchi et al., 2006; Engel et al., 2008). Finally, another limitation was observation (exposure) bias. For example, the accuracy of a producer to classify his/her cattle herd as vaccinated was not assessed in this study. In Nigeria and other countries in sub-Saharan Africa, the mobility and dispersion of nomadic pastoralists present significant financial and logistic constraints to veterinary services for vaccination of livestock.

Overall, the study results revealed that sero-positive cattle herds were associated with exposure to neighbourhoods with FMD activity and by cattle herds sharing water points. Results from this study can be used by veterinary services in Nigeria and neighbouring countries to evaluate current or future FMD control and eradication programmes.

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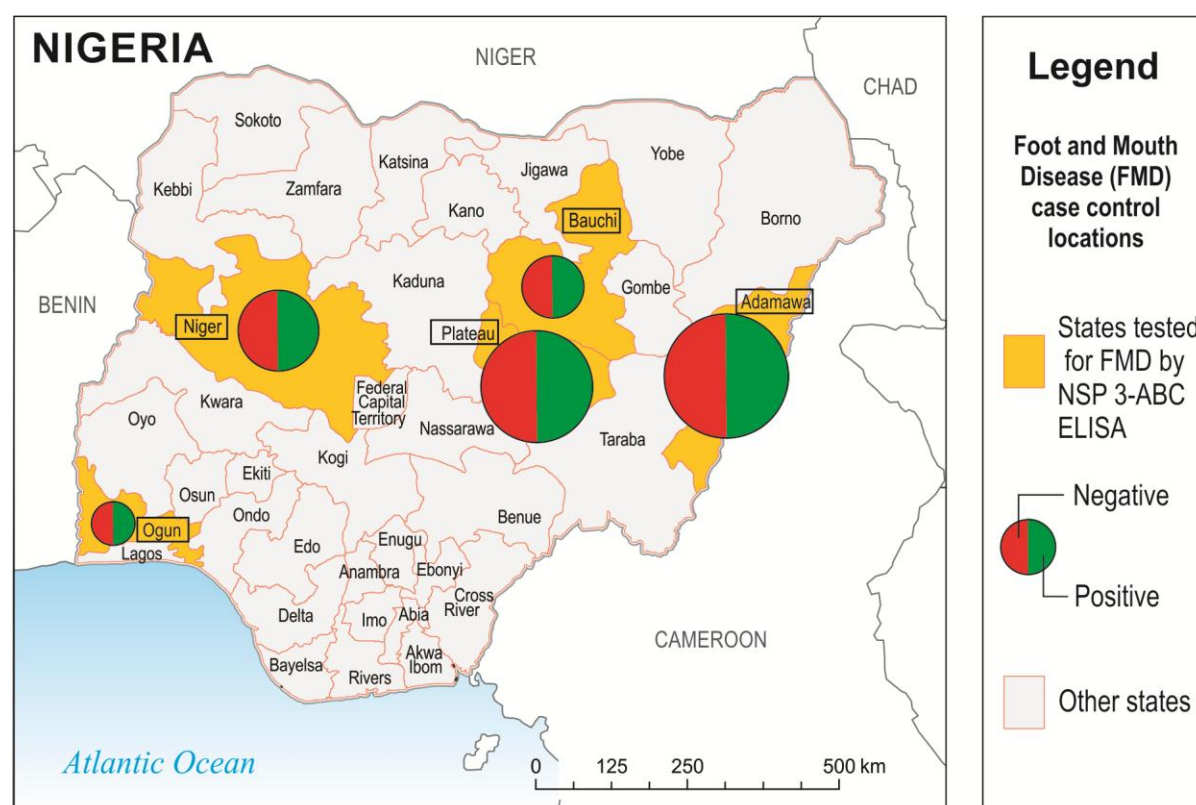


Figure1. States selected for the case control study for sero-positivity to FMD in Nigeria, 2007-2009

Table 1: Multivariable analysis of risk factors associated with foot-and-mouth disease virus infection on cattle farms in Nigeria

Variable	Coefficient	Standard error	Adjusted OR	95% CI	P
FMD outbreak in the neighbourhood	2.7895	0.7680	16.27	3.61, 73.31	< 0.01
Farmer and cattle herd share water points on the trek with other herds	1.4243	0.7687	4.15	0.92, 18.74	0.06

AN OUTBREAK OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN DOMESTIC OSTRICHES: THE CURRENT SITUATION IN SOUTH AFRICA

Van Helden, L.S., Grewar, J.D., Visser, D., Dyason E. and Koen, P.,

BACKGROUND

The ostrich production industry of South Africa is concentrated around two main areas, namely the Klein Karoo valley surrounding Oudtshoorn and the South-Western Cape area surrounding Heidelberg. Several other farms occur in the surrounding provinces, but at the beginning of 2011, the Western Cape housed over half of South Africa's total population of domestic ostriches and 70% of breeding stock. Approximately 240 000 ostriches were present in South Africa at this time; and the industry directly providing 20 000 employment opportunities and contributing over two billion rand to the economy. South Africa was the producer of 70% of the world's ostrich meat, leather and feathers, and 90% of local product was exported, mostly to the European Union (Barnard, A pers comm).

In the last decade, previous outbreaks of highly pathogenic avian influenza (HPAI) H5N2 in ostriches occurred in 2004 in the Eastern Cape and 2006 in the Albertinia region of South Africa¹.

Every ostrich farm in South Africa is part of an avian influenza (AI) surveillance programme in which a relevant sampling frame of ostriches on the farm are sampled for AI testing using serology.. Furthermore, within 28 days of the slaughter of any group of birds, testing of a sample of this group is performed as well.

In the past there have been periods of time where ostrich farms have tested serologically positive against AI during routine surveillance. These farms would be immediately quarantined and samples of all groups of birds on the farm retested using a sampling frame based on a minimum expected prevalence of avian influenza of 5%. This increased sampling event would also include the sampling of birds for AI virus detection using PCR testing. In the past cloacal swabs were the norm for sampling for AIV detection but as a result of consultation with experts from Europe during the outbreak described this has changed to tracheal swab collections from the ostriches. Historically it has been difficult to determine the further characterisation of AI on a farm beyond the limitations of the haemagglutination test (HI) serological results. After epidemiological investigations on the farm indicate no circulating virus and there are two negative rounds of testing 28 days apart, lifting of the quarantine on the farm has been requested. Since 2006, despite PCR testing being performed as a follow-up test on all seropositive farms, no confirmation of active virus circulation could be detected.

METHODS

Evidence of AI was detected in March 2011, when ostriches on five relatively unconnected and geographically distant farms in the Oudtshoorn valley tested seropositive for the H5 subtype of the virus. These properties were placed under quarantine and thoroughly resampled, taking serum samples for HI testing and cloacal swabs for PCR. On 9 April 2011, a highly pathogenic strain of avian influenza (HPAI) subtype H5N2 was identified by the Onderstepoort Veterinary Institute (OVI) from a dead ostrich chick from one of these seropositive properties at, using real-time PCR and RNA sequencing.

Four days later an outbreak operations centre was set up at the South African Ostrich Business Chamber (SAOBC) and attended by representatives from Western Cape Veterinary Services, the national Department of Agriculture, Forestry and Fisheries (DAFF) and the SAOBC. Within the first week of being established, representatives from NICD, Oudtshoorn disaster management, Oudtshoorn Traffic Department, Eden District response teams, the South African Police Service and environmental health began assisting in the outbreak response

Export of fresh ostrich meat from South Africa was immediately halted by DAFF and an avian influenza control area (AICA) was set up using natural boundaries to include the entire Klein Karoo valley, in which there were strict movement restrictions. No movement of live ostriches, ostrich eggs or unprocessed eggshells and unprocessed feathers was allowed into, out of or through the disease control area unless permitted and overseen by state veterinary officials. Surveillance of every ostrich farm in the disease control area was immediately initiated. 30 ostriches from every epidemiological group (separated by age and/or location on the farm) on each farm were sampled by taking serum samples and cloacal swabs. If clinical signs of respiratory disease were noticed, tracheal swabs were taken as well. After the initial round of surveillance, swabs taken were limited to tracheal swabs for all sampled birds.

There was much debate over the case definition of an infected property to be used, but in essence the case definition of an HPNAI infected farm was decided as any ostrich farm in the AICA which tested PCR positive to HPAI H5 or

just to the H5 gene; or with positive H5 serology and positive PCR results for AI matrix gene. A suspect case was defined as any farm from which positive H5 serology results were received. Epidemiological links or geographic proximity between suspect farms and positive farms also played a role in determining the final status of a farm. The presence of clinical signs on the farms was included in the case definition but did not play a large role in identifying infected properties, as the majority of positive farms did not show an increase in morbidity or mortalities of ostriches.

Birds on farms where active circulating virus was identified by PCR were slaughtered at the Klein Karoo abattoir in Oudtshoorn. Feathers and skins were salvaged from birds that were large enough, and the remainder of the carcasses were rendered to make carcass-meal. These farmers received compensation from DAFF for the lost value of the ostriches. Birds from farms which were seropositive only were also depopulated by slaughter with compensation; however, the breeder birds on these farms were allowed to remain where they were. Tracing of movements on and off all suspect and positive farms was performed and all connected farms were followed up by sampling and testing. Accelerated surveillance of farms outside the AICA was begun in May 2011 and all ostrich farms in the Western Cape had been sampled at least once by August 2011. Over the outbreak period thus far approximately 513 individual properties were visited and sampled by state officials.

RESULTS

A total of eight rounds of surveillance took place inside the AICA between April 2011 and February 2012. A total of 20 infected farms and 20 suspect infected farms were uncovered up to November 2011, 14 of which were confirmed HPAI H5N2 positive using RNA sequencing of the H5 cleavage site. Interpretation of serology results was confounded by several concurrent H6 infections. However, the final picture revealed two clusters of infection in the Volmoed and Buffelsdrift areas south-west and north of Oudtshoorn respectively. Both of these areas had proportionately dense populations of ostriches before the start of the outbreak.

Two positive (confirmed HPAI) and one suspect (H5 seropositive with trace links to the Klein Karoo valley) positive properties were detected outside the control area between April 2011 and February 2012. These properties were slaughtered out and a 10km zone around each was set up and all the farms in this area quarantined and sampled.

An approximate total of 37 000 birds were slaughtered and 3 000 eggs destroyed as a direct result of the outbreak, representing a loss of 15% of the country's total domestic ostrich population. Just less than 53 million rand has been spent to date by national and provincial veterinary services in compensation alone.

The disease control area in the Klein Karoo was officially dissolved in March 2012, after two full rounds of surveillance inside the AICA had returned only negative results.

Unfortunately, however, before South Africa could be in the position to apply to have its export status reconsidered by the EU, highly pathogenic H5N2 AI was identified on a previously H7-positive farm in the Heidelberg area in June 2012. This outbreak is currently being handled using the principles developed in Oudtshoorn. This outbreak currently consists of three properties in the Southern Cape, one of which has been slaughtered out.

CONCLUSIONS

The beginning of the outbreak was a steep learning curve for all officials involved, dealing with problems for which there were not necessarily contingency plans, such as limited capacity of samplers and data processors, adverse weather conditions, dangerous working conditions and the ability of available laboratories to process large numbers of samples urgently.

Much debate over how to control the occurrence and spread of avian influenza has taken place, and unfortunately there are large gaps² in scientific knowledge of this disease in ostriches. It has furthermore been previously established that a reservoir of H5N2 is established in the wild duck population of South Africa¹. As a result, limited recommendations for disease control can be made beyond those that are already known. Negotiation between state officials and the SAOBC is constant in an attempt to create an environment within the industry that is sustainable whilst still maintaining high levels of biosecurity and control of ostrich and ostrich product movement.

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AN OUTBREAK OF AFRICAN HORSE SICKNESS IN MAMRE, SOUTH AFRICA DURING 2011.

Weyer, C.T.¹, Grewar, J.D.², Guthrie, A.J.¹, Davey, S.² & Buhrmann, G.²

SUMMARY

African horse sickness (AHS) is a controlled disease in South Africa. It is a life threatening disease of equids caused by African horse sickness virus (AHSV), a member of the genus *Orbivirus* in the family *Reoviridae*. The virus is transmitted to horses by midges (*Culicoides spp.*) and the disease is most prevalent during the time of year, and in areas where the *Culicoides spp.* are most abundant, namely in late summer in the summer rainfall areas of the country (Coetzer and Guthrie (2004)). The area around the Cape of Good Hope in South Africa has historically been free from AHS (Guthrie (2007)). An outbreak of AHS serotype 1 occurred in the AHS surveillance zone of the AHS controlled area of the Western Cape in the summer of 2011. The outbreak started in the town of Mamre in the magisterial district of Malmesbury, and was effectively confined to this area by movement control of all equids and a blanket vaccination campaign of the immediate outbreak area. Of the total 73 confirmed cases of AHS that occurred during this outbreak a small number were found to be subclinical cases with the horses showing no outward clinical symptoms. The source of this outbreak was never confirmed although it is thought to have occurred due to illegal movement of an infected animal into the surveillance zone.

INTRODUCTION

The first confirmed case was reported on Saturday the 26th February 2011. The state veterinarian (SV) of Malmesbury was called by a private veterinarian to assist in a post mortem (PM) of a horse that had died showing symptoms suspicious of AHS in the Mamre area in the Western Cape. The PM signs correlated with a possible AHSV aetiology and blood and tissue samples were taken and sent to the Equine Research Centre (ERC) at the University of Pretoria (UP) for AHS testing. The ERC currently uses a group specific real-time RT-PCR targeting the genes coding for the VP7 protein of AHS to quantify the viraemia (Quan, Lourens, Maclachlan, Gardner and Guthrie (2010)). Samples were also later sent to the Onderstepoort Veterinary Institute (OVI) for testing. On the same day the state veterinarian sampled a dead horse found next to the road in the Mamre area. On Wednesday the 2nd of March 2011 another horse was reported dead by an owner from Mamre. A post mortem was performed on the same day and PM results were highly suspect for AHS infection. Tissue samples were taken and sent up to both laboratories mentioned above. On the same day the results for the initial two horses sampled were received from the UP laboratory with positive PCR results for AHSV. On the 7th March 2011 the OVI results were also received from the same horses and were positive for AHSV and negative for equine encephalosis virus (EEV). On the 4th March 2011 results from the third horse sampled were received from UP also indicating positive AHSV and this was later confirmed by OVI on the 7th March 2011. The test results and PM signs at this point in the outbreak prompted the reporting of the outbreak to the Department of Agriculture, Forestry and Fisheries (DAFF). Implementation of control measures and plans to attempt to contain the outbreak were initiated by the provincial veterinary services.

CONTROL MEASURES

Control measures were instituted in consultation with the private veterinarians of the Malmesbury district. Advice was given to horse owners to stable their horses from 2 hours before sunset to 2 hours after dawn to decrease the risk of the vector contact with their horses. Owners were also advised to make use of a registered insect repellent on their horses during the vector feeding periods as indicated above. Further advice to owners via their private veterinarians was to ensure that their horses' AHS vaccinations were up to date.

A movement ban was imposed which limited movement of horses into, within and out of the Malmesbury magisterial district. The Malmesbury district was placed under quarantine for the movement of horses on 3 March 2011 by means of a press release, and this quarantine was lifted three months later on 9 June 2011

Ring vaccination within the immediate vicinity of the initial cases in Mamre was also initiated. This vaccination was limited to AHS bottle one after the serotype of the AHSV was confirmed as serotype one by UP on the 9th of March

1 Equine Research Centre, Faculty of Veterinary Science, University of Pretoria

2. Western Cape Department of Agriculture, Republic of South Africa

2011. There were many horses in the Mamre area which were fully susceptible to AHS infection at the start of the outbreak as they had not previously been vaccinated against AHS. The ERC from the UP and the Western Cape Department of Agriculture provided the OBP polyvalent AHS vaccine for the developing farmers in the Mamre area and surrounds, and a total of 447 horses were vaccinated, with the majority being in early March 2011, approximately 1 week before the outbreak peaked. A dramatic decrease in the number of cases seen per week was seen 2 weeks after the majority of the horse population in Mamre had been vaccinated. The majority of horses vaccinated were within the Mamre town limits (n=289). This accounted for 64.6% of total vaccinated animals and this covered approximately 90% of the equines within Mamre. Reports from the Malmesbury district show that 189 horses were vaccinated by private veterinarians during the outbreak period. This brought the total of vaccinated equines during the outbreak period to approximately 636.

QUANTIFICATION OF THE OUTBREAK

A unique feature with regards to the handling of this outbreak was the surveillance methods employed. As far as was practical EDTA blood samples were taken from each horse presented to the outbreak response personnel prior to vaccination. This ensured that active surveillance on a census level sampling frame was accomplished and this provided a dataset from which accurate determination of epidemiological variables could be made as testing results improved the accuracy of confirmed negative cases in the population at risk.

The confirmed AHS cases totalled 73 horses. There were a total of 16 suspect cases, with horses in the suspect category based on the case definition (see Table 1). Mamre town itself is contained on a zoned piece of land approximately 3900 Ha in area. This is where the most complete census and investigations were performed and 77% (n=56) of the cases occurred in this area. The census in this area totalled 319 equines and, assuming every censused equine was susceptible, the incidence of AHS in this area was 0.17 (95% Conf. 0.13-0.22) during the outbreak period. The total AHS related deaths recorded during the outbreak (both AHS confirmed (n=64) and suspect cases (n=6)) was 70 horses. The majority of these deaths impacted previously disadvantaged owners.

There were a number of suspect (n=11) and confirmed (n=4) subclinical cases recorded in this outbreak, which is also a unique feature for an outbreak in the surveillance zone, but has been described recently in endemic areas (Weyer, Quan, Joone, Lourens, MacLachlan and Guthrie (2010)). A subclinical case was defined as an animal that was found to be RT-PCR positive with a Ct lower than 30, with or without positive AHS viral isolation (VI) results, but showing no discernible clinical signs of AHS, and otherwise healthy. AHSV serotype 1 was isolated from 4 of these cases. The other 11 suspect subclinical cases were PCR positive, some for an extended period of time, but we were unable to isolate virus from the blood samples. Of these 11 cases, 8 were sampled prior to vaccination, so the positive RT-PCR result was most likely as a result of field infection not vaccine. The other 3 were sampled between 1 and 2 weeks after vaccination, so the positive result may have been due to vaccination or field infection. There was one other remaining suspect case, which was not classified as subclinical as the horse died. However, although the horse was found to be RT-PCR positive, the PM findings were not typical of AHS, the sample was found to be negative on VI, and the horse's vaccination history was unknown. It was therefore decided to classify this horse as suspect rather than confirmed.

Table 1: A table showing the case definition codes categorising the cases according to the clinical signs and results of each case, and the numbers of cases in each category.

Case Definition	Code	Definition	Number of cases per case definition code
Negative	N1	Clinical signs and/or PM signs similar to that of AHS with confirmation of another cause of disease or confirmed negative for AHS on laboratory results	14
	N2	Routine testing for surveillance with negative AHS laboratory results	158
Positive	P1	Clinical signs and/or PM signs of AHS with laboratory PCR and/or VI positive result	69
	P2	PCR <u>and</u> VI AHS positive result with no accompanying clinical or PM signs	4
Suspect	S1	Clinical signs and/or PM signs of AHS with no laboratory positive confirmation	4
	S2	No clinical or PM signs of AHS with PCR result of CT value ≤ 30 - VI negative	12

VECTOR SURVEILLANCE

Down-draft, 220 V light-traps equipped with 8 W UV-light tubes were used for *Culicoides spp.* collections. One light trap was set up in the town of Mamre near the stream that runs through the town, where most of the horses are kept in the town, and one trap was set up at Pine Ranch, which is where one of the outlying cases occurred. The first sample collection was on 11 April 2011. Each collection started at around 6pm each evening until around 7am the following morning. Daily collections were intended to be taken for the first 5 days and then twice weekly thereafter until the end of the vector season (June). The owner of Pine Ranch was responsible for that collection point, and a horse owner in Mamre that lived next to the collection site was chosen to be responsible for the town collection point.

Operator compliance was a problem in Mamre, and that may have affected the numbers that were obtained from the traps set up in Mamre. The traps were not always set up at the correct times, occasionally collections were not taken at all on the days specified, and after 2 weeks the electricity cable supplying the light trap was stolen, so no further collections were taken from Mamre. Although the Pine Ranch collections were not always taken to the exact schedule given, operator compliance was better, and a more accurate sample collection was attained.

As the collections from Mamre were erratic it was not possible to see any trend with regards to population numbers. The Pine Ranch collections showed a dramatic drop in numbers one week after collection started, and had dropped even further to negligible numbers by the beginning of June. By far the majority of collections taken at Pine Ranch consisted of *C. imicola* as the dominant species which was to be expected. However, the more dominant species in each collection in Mamre was *C. subschultzei*.

DISEASE SPREAD

By far the majority of cases were considered to be as a result of local infection circulating in the *Culicoides* population and spread via movement of infected horses. Demographics of the situation meant that it was initially difficult to prevent the movement of infected animals within the Mamre area and immediate surrounds, as there are no formal horse societies to advise and pressurize owners, and no incentive not to move horses as per normal. It was found that personal communication with each individual horse owner in Mamre and the immediate surrounding areas was necessary in order to explain the reasons for movement restrictions. This was one of the biggest confines in control of this outbreak compared to past outbreaks of this disease which occurred within wealthier socioeconomic areas where formal stabling and management existed. The movement control strategies into, out of and through the quarantine zone were effective in preventing formal movements via vehicles, which was easier to control as there was cooperation from traffic officials and reporting from other concerned horse owners. This effectively limited the extent in which the infection spread.

DISCUSSION

As AHS is a vector borne disease one would normally expect (if no counter measures are taken) multiple case peaks during an outbreak, however except for a very small peak at the end of April the outbreak in Mamre only showed one peak in disease cases. This is most likely due to vaccination in the Mamre area and particularly in the surrounding areas like Pella and Atlantis which prevented significant seeding of the outbreak.

The source of the outbreak could not be confirmed, although investigations by the SV did show that there were suspected illegal movements of horses into the AHS surveillance zone, which were a risk for the introduction of AHS into the area. The various equestrian societies which rely on movements of horses for their various events must ensure that horses participating have undergone the correct procedures when entering the various AHS control zones.

Past outbreaks of AHS in the surveillance zones have relied on serology to define the spread of the disease rather than molecular methods. Here EDTA samples were taken and the RT-PCR used currently at the ERC was used to quantify the spread of AHS within the population. This method gives a more accurate impression of a point epidemic, and previous exposure would not interfere with interpretation of results as it does with serology. The cycle threshold (Ct) is the number of cycles taken before fluorescence occurs. For the purposes of this outbreak, samples tested with the RT-PCR used at the ERC were classified as positive if the fluorescence exceeded the threshold of 0.1 within a maximum of 30 cycles. Suspect AHS cases that showed no typical AHS symptoms and had a Ct value over 30 were classified as negative for AHS.

The subclinical cases found in this outbreak, although substantially fewer than the clinical cases, are still a possible cause for concern when considering spread of disease. This is further concern when you consider that these cases, without the aid of molecular methods such as the RT-PCR, would not be diagnosed, as the relevant samples would not be taken as the horse would not show clinical signs. These cases may only have had serum samples taken, and then diagnosis would not have been made as the horse would have been vaccinated in the face of an outbreak, and the serological responses found would have been considered to be as a result of vaccination. Further, when you consider that of the 15 subclinical cases found, only 4 also had positive isolation results, it shows the sensitivity of the PCR employed in comparison to the historical isolation methods.

This outbreak highlighted the importance of partnerships in the control of diseases such as AHS. Private-public partnerships between members of Equine industries and State Veterinary Services ensured a rapid response to this outbreak, and the State was able to start vaccinations immediately with the AHS vaccine donated by the ERC, and the personnel of the different bodies were able to work together in an efficient manner. This also helped in building a relationship with the community of Mamre, as all parties involved were seen to have the same objectives and the best interests of the community at heart.

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INVESTIGATION AND CONTROL OF AN OUTBREAK OF AFRICAN SWINE FEVER IN THE GAUTENG PROVINCE IN 2012.

Geertsma, P. J., Mpofu, D. & Walters, J.

ABSTRACT

Certain north eastern areas of South Africa are considered endemic for African Swine Fever. The cycle in South Africa has classically been a outbreak associated with Warthog (*Phacochoerus aethiopicus*) and bush pig (*Potamochoerus porcus*) as inapparently infected then vertical transmission to domestic pigs through Tsetse flies (*Ornithodoros moubata*).

This paper describes a horizontally transmitted outbreak outside of the legislated Control Area for African Swine Fever (Animal Diseases Act, 35 of 1984) with subsequent spread to 6 properties within Gauteng province. The outbreak covered three provinces, resulting in a coordinated control effort between provincial administrations. The actions taken in the outbreak with forward and backward tracing, slaughter out and laboratory testing are discussed. Lessons learnt from the outbreak will be presented leading to increased awareness and control of possible similar outbreaks in future.

INTRODUCTION

African swine fever (ASF) is a highly contagious hemorrhagic disease of pigs that produces a wide range of clinical signs and lesions that closely resemble those of classical swine fever. In South Africa the normal picture is sporadic outbreaks seen in the declared ASF control zone of the Northern provinces (See map below). These outbreaks occur with either mixing of warthog and/or bushpig with domestic pigs or feeding of offal from hunted warthog and/or bushpig being fed to domestic pigs. The disease can be either peracute, acute or chronic with mortality rates from 0 to 100 %. In South Africa the mortality rate is usually higher with the peracute or acute form of the disease seen. These outbreaks are controlled with culling of all surviving pigs at the time of detection. If the farmer has not observed the control measures as they apply to keeping of pigs in the ASF control area, then no compensation is paid. Of a total of 29 outbreaks since 1993 (See Table 1 below), two have been outside of the ASF control area since 1993⁽¹⁾. Both of these have been adjacent to the border of the control area. The last outbreak of ASF outside of the ASF controlled area occurred in 1996 in Bela-Bela with an illegal movement.

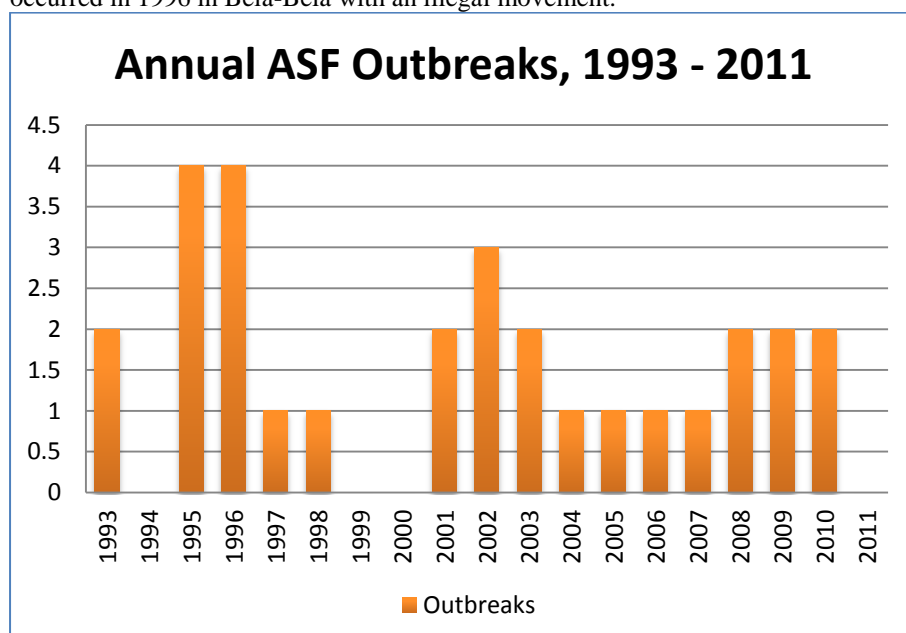


Figure 1: Histogram showing ASF outbreaks in South Africa from 1993 to 2011

OUTBREAK EVENTS

A chicken and meat wholesaler bought pigs from the Sundra Auction (Trio Auctioneers) between the 26th November 2011 and 11th December 2011 with the intention of fattening and slaughtering them. However, some of the pigs

started getting sick and showed signs of respiratory infection. After showing no response to treatment with terramycin, the wholesaler consulted a local private veterinarian who advised them to treat the pigs with Penicillin. On the 23rd December 2011, some of the pigs started dying and 2 carcasses were taken to a private veterinarian in Benoni. Pneumonia was diagnosed by a local laboratory and this is considered to be unrelated to the outbreak. However, the pigs continued dying and to minimize losses, the wholesaler decided to slaughter them all and arrangements were made with Reitpoort Abattoir for slaughtering to be done on the 3rd January 2012.

Reitpoort Abattoir, located in Nigel, Gauteng, received 52 pigs for slaughter from the wholesaler on Tuesday 3rd January 2012. 28 pigs were slaughtered and the meat inspector identified lesions that highly resembled Classical Swine Fever (Hog Cholera) or African Swine Fever (ASF). A private veterinarian and an AHT in Gauteng area went the following day and made a presumptive diagnosis of ASF from the lesions they observed. The Transboundary Animal Disease Program of the Onderstepoort Veterinary Institute diagnosed ASF on PCR and subsequently isolated the ASF virus from a set of pooled organs. All 52 pig carcasses from the batch were subsequently destroyed and buried.

On 11th January the State Vets from Mpumalanga quarantined the Trio Auction pens. The owner said that he had had 120 mortalities and had taken all of his remaining pigs to the abattoir. He had purchased from 9 different owners at the Trio Auction. These owners were followed up.

A Joint Meeting was held between Mpumalanga, Gauteng and the Department of Agriculture, Forestry and Fisheries (DAFF) where it was decided to close all auction pens in both provinces until the outbreak was resolved. This was to stop the informal trade in pigs and thereby prevent further spread of the virus. A Veterinary Operational Committee was established in Gauteng to coordinate the response.

A movement protocol was established for all pig and pig product movements. Only trade to an abattoir was allowed and this if veterinary services was sure there was no infection on the farm. These movements were via red-cross veterinary movement permits. All farms that had traded through the auction pens which had been identified as having had a role in the spread of the disease were placed under veterinary quarantine. These farms were then inspected every 10 days to identify any increase in mortalities.

At a joint meeting with the South African Pork Producers Organisation (SAPPO), industries assistance was sought to speedily curtail the spread of the disease. SAPPO then offered to pay for feed of pigs that were kept informally and roamed outside of pens for foraging. These pigs were then penned to prevent spread of the disease. SAPPO also offered to purchase the surviving pigs from each infected farm so they could then be slaughtered. This was to get full and rapid compliance from infected premises owners.

A focus of infection was discovered at Withok (11 January) followed by another one at Palmietfontein. The third focus was at Nooitgedacht in Devon. Forward and backward tracing was undertaken to and from two auction pens, Trio and RMB in Sunda and a third in Bronkhorstspuit later on. The outbreak involved both Mpumalanga and Gauteng Provinces. Pigs were dying slowly on the infected farms. The inspection of pig keeping facilities continued for surveillance purposes.

Two properties on the farm Vischkuil (2nd February) close to each other had also been placed under quarantine during follow-up investigation after mortalities were reported. Postmortem examinations indicated ASF and the diagnosis was also confirmed with positive PCR results and virus isolation.

A focus of infection was discovered (22nd January) on Schietpoort, Kungwini. The pigs were bought at the Bronkhorstspuit auction from a farmer in Mpumalanga. All 7 pigs on this property died and were buried on the property.

As soon as the go ahead was given by SAPPO to pay for the live pigs and the farmers had all signed contracts with SAPPO on the terms agreed, the culling was planned. The Society for Prevention of Cruelty to Animals was present at the culling. All pigs were stunned using a captive bolt and then given an overdose of pentobarbitone intra-thoracically.

The virus has been phylogenetically traced to an ASF virus from Limpopo province. The closest link is to a virus from the Lephalale municipality⁽²⁾. There was an illegal movement of pigs from a farm in that area in November, 2011. This was followed up by the local State Vet who found that there had been mortalities on the farm. The owner had then decided to sell pigs in Gauteng. According to the owner his mortalities has stopped for 2 weeks prior to selling. This is the most probable source of the infection. The remaining pigs on that farm were bled and tested with the ELISA test. One pig was found to be sero-positive for ASF. This pig was culled and sent to the provincial laboratory for post mortem. No virus or even PCR positive result was found on the farm. Tampans collected subsequently on the

farm did show a positive ASF PCR on 1 out of five warthog burrow complexes' samples collected. No virus isolation was possible from this sample so no phylogenetic matching was possible

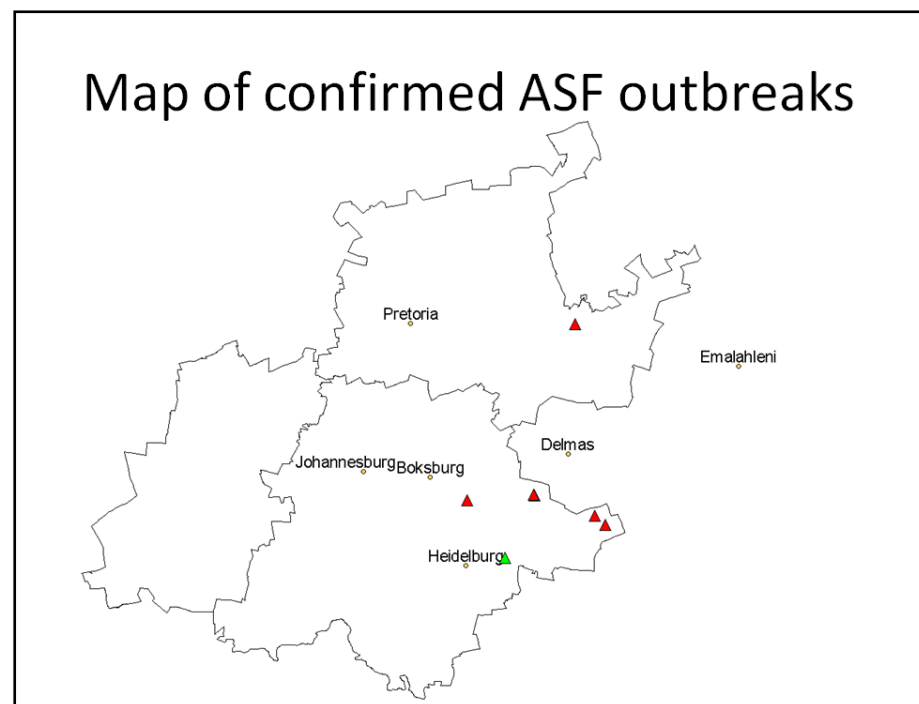


Figure 2: Map of Gauteng province showing confirmed outbreaks.

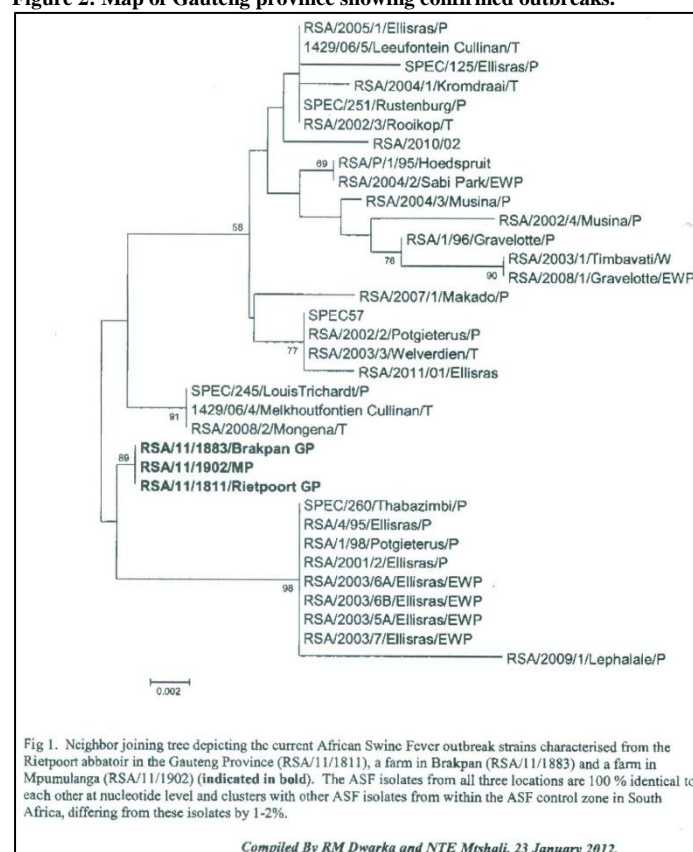


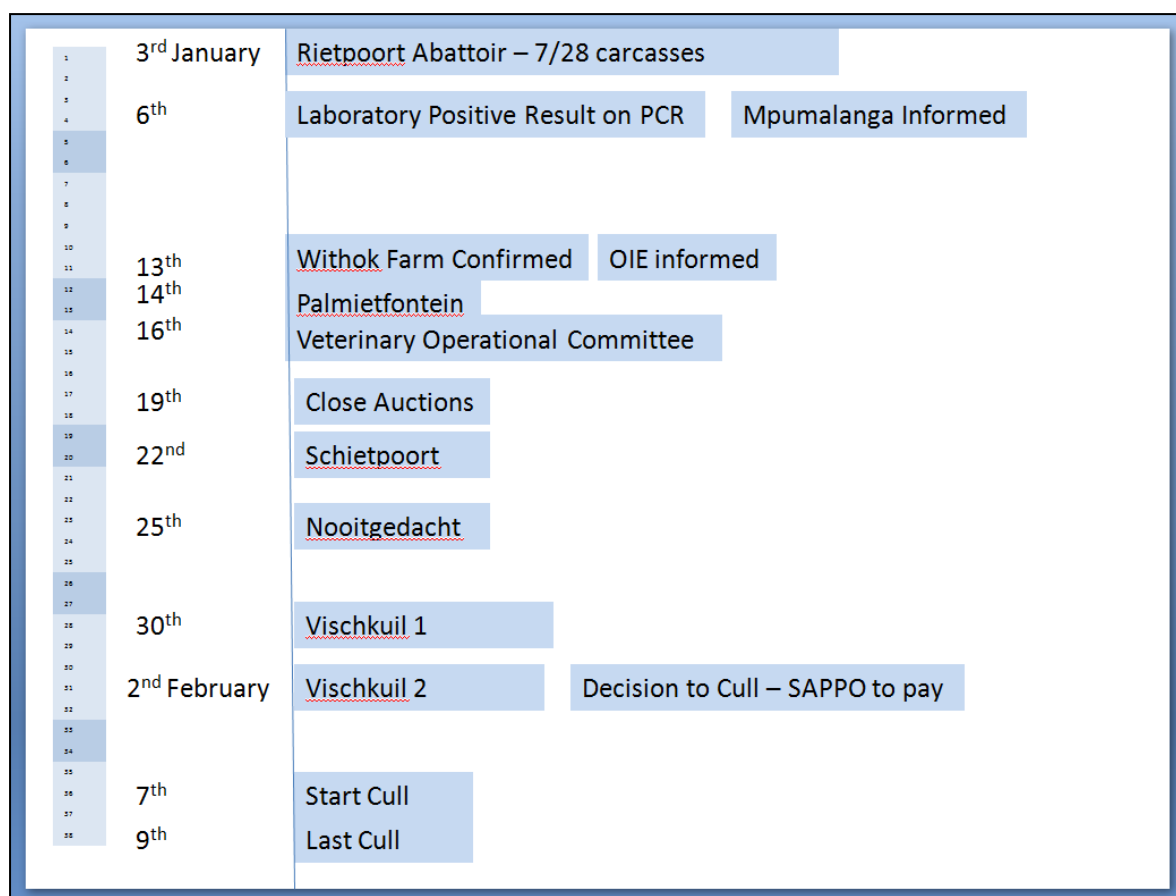
Figure 3: Phylogenetic tree showing relation of outbreak virus to previous RSA isolates

Table 1: Gauteng infected premises showing mortalities and eventual numbers culled.

Farm name	Farm ID	Municipality	Animals Susceptible	Number Quarantined	Cases per SR1	Death per SR1	Piglets Born Since Quarantine imposed	Deaths since Quarantine imposed	Culled
Germiston State Vet									
Withok AH	131 IR	Ekhurhuleni	117	64	61	53	0	20	44
Palmietfontein	316 IR	Lesedi	108	75	38	33	22	9	88
Nooitgedacht	294 IR	Lesedi	9	6	5	3	0	4	2
Vischkuil	274 IR	Lesedi	39	39	17	15	0	10	14
Vischkuil	274 IR	Lesedi	17	13	5	4	4	0	17
Pretoria State Vet									
Schietpoort	507 JR	Lesedi	10	7	5	3	0	7	0
Total			300	204	131	111	26	50	165

RESULTS

All hundred and sixty five (165) pigs on the other five infected properties in the Gauteng were destroyed and disposed of in a biologically secure manner over two days (7-8th February) at the Platkop landfill disposal site. Under veterinary supervision, the carcasses were trenched, limed and covered over with soil to avoid scavenging.

**Figure 4: Timeline of events in the outbreak.**

The pig sties on the 6 infected properties were disinfected twice, after culling and again on/ before the 13th of April under State Veterinary supervision with chlorides and quaternary ammonium compounds.

Owners of the pigs were paid R250.00 per pig less than 50kg live weight and R450.00 per pig more than 50kg live weight – a contract with SAPPO in lieu of state compensation.

All 13 public livestock auctions in Gauteng were also disinfected under SV supervision as pig marketing had been suspended to allow for tracing and determination of extent of disease.

A reminder was sent to all livestock auctioneers to comply with Animal Disease Act in terms of not accepting pigs from the ASF control areas as well as the Animal Identification and Stock Theft Acts.

All quarantined sites with pigs (57 properties with 2474 pigs) linked with the auctions Trio, RMB and the Bronkhorstspuit auction were inspected once in ten days to ensure the investigation of all possible new mortalities. The quarantine was lifted 60 days later at the end of March 2012.

The prohibition order on the trade of pigs at Gauteng livestock auctions was lifted during the last week of March 2012 after the auctioneers undertook to put in place biosecurity measures and comply with the Animal Identification Act as well as the Stock theft Act.

DISCUSSION

This is the first large scale outbreak of ASF outside of the control area. It is the first time South Africa has experienced lateral transmission in the absence of the tsetse vector. The outbreak involved two provinces and although the source has yet to be identified definitely, it is assumed to be from an illegal movement from the ASF control area.

A number of lessons were learnt during the outbreak and include, *inter alia*:

Better overall preparedness for such a situation which will allow for a more rapid and concerted effort, including purchases, compensation, liaison between provinces and the national government and revising of general contingency plans;

Improved communication especially with the farming community and organised agriculture to allow more free movement of important information;

The human element in a massive culling operation like this needs to be addressed from the start as soon as the decision to cull is taken;

Animal welfare concerns are always to be taken into consideration in such an operation.

The trade in pigs on these informal markets has come under veterinary scrutiny and has led to this trade being better controlled through the implementation of existing legislature around animal identification, stock theft and disease control. The detrimental effect on this market through the outbreak has been restored and all restrictions were able to be lifted in Gauteng by the end of March.

The rapid control and eradication of the disease in Gauteng has been a major achievement by the veterinary services. Without the prompt assistance of SAPPO this would not have been the case and the disease may have become endemic in the area with resultant detrimental consequences to the local industry and international trade.

ACKNOWLEDGEMENTS

We would like to acknowledge the Transboundary Animal Disease Program's Dr R. Dwarka and NTE Mtshali for their use of the phylogenetic tree and Gauteng Department of Agriculture and Rural Development for their assistance with this paper.

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RISK FACTORS FOR AFRICAN SWINE FEVER AND THE EFFECT OF BIOSECURITY

Fasina^{1,2*}, F. O., Agbaje³, M., Ajani³, F. L., Thompson¹, P. N. and Bastos², A. D. S.

SUMMARY

ASF is economically devastating for the pig industry. Data on risks of ASF are lacking from West Africa. Evaluation of risk factors supporting infection of pig farms in this region remains the key to the development of a risk-based approach to the epidemiology of ASF and control. In Nigeria, perpetual infections of certain localities with intermittent infection of contiguous areas makes it an ideal setting for a matched case-control study for risk factors and biosecurity practices in pig farms. Subsets of farms located in high-density-high-risk pig areas for ASF infection were randomly selected for this analysis. The presence of abattoir within a pig farming community (OR = 8.20; CI_{95%} = 2.73; 24.63; $P < 0.001$); and the presence of an infected pig farm in the neighbourhood (OR = 3.26; CI_{95%} = 1.20; 8.83; $P = 0.02$) were significant. There was a marginally significant negative association (protective) between risk of ASF infection and sharing of farm tools and equipment (OR = 0.35; CI_{95%} = 0.12; 1.01; $P = 0.05$).

Of the 28 self-biosecurity measures evaluated, food and water control (OR = 0.14; CI_{95%} = 0.04, 0.46; $P < 0.001$), separation of sick pigs (OR = 0.14; CI_{95%} = 0.04, 0.53; $P = 0.004$) and washing and disinfection of farm equipment and tools (OR = 0.27; CI_{95%} = 0.10, 0.78; $P = 0.02$) were negatively associated (protective) with ASF infection. Consultation and visits of veterinarian or paraveterinarians when animals were sick (OR = 8.11; CI_{95%} = 2.13, 30.90; $P = 0.002$), and pest and rodent control (OR = 4.94; CI_{95%} = 1.84, 13.29; $P = 0.002$) were positively associated with ASF infection of farms in Nigeria.

Consideration of these factors should inform policy formulation to control ASF regionally in West Africa. Farm-based biosecurity is also important as it will benefit ASF control and cover other infectious diseases in the sub-region.

¹ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.

² Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa.

³ College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria.

* **Contact details of corresponding author:** Department of Production Animal Studies, University of Pretoria, Onderstepoort 0110, South Africa. Telephone: +27 12 529 8069; Fax: +27 12 529 8396; Email: dayo.fasina@up.ac.za

THE BRUCELLIN SKIN TEST: IS IT OF ANY VALUE TO INVESTIGATING BOVINE BRUCELLOSIS IN SOUTH AFRICA?

Nyanhongo, N., Hansen, M., Storm, A. & Michel, A.L.

SUMMARY

Brucellosis is an international disease of high economic impact besides being a significant zoonosis. Veterinary efforts at control and eradication are hampered by its propensity for long, and at times latent, incubation periods when the widely used serological methods are unable to identify carriers. No single test is able to identify all stages of the disease with high sensitivity. The brucellin skin test (BST), which has been proved to identify some acute and chronic latent stages of brucellosis, has not been validated in South Africa. The objective of this study was to evaluate the BST in brucellosis free, as well as brucellosis infected cattle herds, under South African farming conditions. The results indicate that although the BST has a relatively low sensitivity of 53.97%, it has a high specificity of 99.01%. It was able to detect more cases in infected herds when compared to the routinely used Rose Bengal and Complement Fixation tests. It was concluded that BST could be used in parallel with serological tests to improve the sensitivity of the current diagnostic regime.

INTRODUCTION

Brucellosis, due to infection by gram-negative bacteria of the *Brucella* spp, is a disease of socio-economic and zoonotic importance worldwide. In animals it is associated with the ingestion of feed that is contaminated with cyetic material from aborting herd-mates while in humans it is associated with the consumption of unpasteurised milk (including products there-from) of infected animals. It may also be acquired from getting into contact with infected material of animal origin as with farmers, veterinarians, abattoir and laboratory workers. A report by Strachan in 1906 (Schrire (1962)) indicated the presence of brucellosis in South Africa in the late nineteenth century. It is still present in the country today, with reported annual losses of at least R300 million, and a national annual incidence of 5000 in humans at certain periods (Schrire (1962), Hesterberg, Bagnall, Perrett, Bosch, Horner & Gummow (2008)). The global incidence of human brucellosis is about half a million infections annually. As the incidence of human brucellosis is directly associated with animal prevalence, control of animal brucellosis is emphasised.

However, veterinary control is compromised by the chronic nature and the variable and often inapparent incubation period of the disease. It is estimated that up to 15% of cattle in infected herds abort before sero-conversion. Latency, which involves about 5% of calves born from infected dams, is another complicating factor as these infected animals usually test negative on routine serological tests only to seroconvert in the peri-parturient period. This characteristic of the disease offers opportunity for disease spread within and between herds before diagnosis is made. In addition, the currently used serological tests are at times unable to distinguish brucellosis from cross-reacting antibodies from other infections or brucellosis vaccines.

It was the objective of this study to investigate, under South African conditions, the value of the brucellin skin test (BST) in improving the sensitivity and specificity of the currently used serological tests. It has proved a valuable additional test in diagnosing early and latent infections as well as in differentiating brucellosis from cross-reacting organisms in unvaccinated cattle in Europe (Bercovich, ter Laak & van Lipzig (1992), Pouillot, Garin-Bastuji, Gerbier, Coche, Cau, Dufour & Moutou (1997)).

MATERIALS AND METHODS

ANIMALS

The study was carried out on fourteen herds and involved 1454 head of cattle in the districts of Lekwa, Dipaleseng, Msukaligwa, Emalahleni, Govan Mbeki and Steve Tshwete in Mpumalanga Province (South Africa). In order to minimise the effect of maternal as well as vaccine antibodies on the tests, only calves between the ages of three and nine months (before they were vaccinated for brucellosis), were selected. The herds were selected to be representative of farming systems in South Africa. The brucellin skin testing, as well as blood collection, was carried out on the farms between November, 2010, and August, 2011, while the serological tests were conducted between December, 2011, and March, 2012, at the Onderstepoort Veterinary Institute (OVI) for the RBT (Rose Bengal test) and CFT (Complement fixation test), and at the Department of Veterinary Tropical Diseases, University of Pretoria, for the iELISA(indirect enzyme linked immunosorbent assay).

BRUCELLOSIS NEGATIVE CONTROL HERDS

There were 608 calves/heifers from five herds on four brucellosis negative farms. They consisted of 155 mixed breed, 430 Drakensburger, and 23 Holstein-Friesland calves. The farms were certified brucellosis-free in accordance with the official Bovine Brucellosis Scheme (Animal Diseases Act, 1984, Bovine Brucellosis Scheme- Section 10) with regular testing (MRT(milk ring test),RBT and CFT). The herds had traceable records as kept by the Department of Agriculture, Forestry and Fisheries in Mpumalanga Province. The minimum certified period of freedom was three years.

BRUCELLOSIS INFECTED HERDS

A total of 846 animals, all from known infected herds, were tested. They consisted of 411 mixed breed and 435 Drakensburger. The herd infection status was based on routine serological testing, supported by, at least one bacteriological, isolation of the *Brucella spp* organism.

SERUM

Approximately 10 ml of blood was collected from each test animal by venipuncture of either the jugular or the median caudal vein into Vacutainer™ tubes without anti-coagulant. The blood was allowed to clot and the serum separated and stored at -20°C until the time of testing.

TEST METHODS

BRUCELLIN SKIN TEST (BST)

The procedure was carried out as described earlier (Saegerman, Vo, de Waele, Gilson, Bastin, Dubray, Flanagan, Limet, Letesson & Godfroid (1999), OIE Terrestrial Manual (2009)). An area of approximately ten square centimetres of healthy skin on the side of the neck was clipped with a pair of scissors and the measurement of normal skin thickness taken with a springmeter (Hauptner). With the aid of a tuberculin syringe coupled to a 4mm, 25 gauge needle, 100 µl of brucellin (batch 10 0001,MEGACOR diagnostic, Austria) was injected intradermally to leave a visible pea-sized nodule at the injection site. The reaction was assessed approximately 72 hours post-injection primarily by sight and palpation followed by measurement with a spring meter. A positive reaction was assessed qualitatively as either a firm well circumscribed induration or as a soft oedematous induration. The same operator took both pre and post injection measurements to minimise variation.

ROSE BENGAL TEST (RBT)

The procedure was carried out at Onderstepoort Veterinary Institute (OVI) as described earlier (Alton, Jones, Angus & Verger (1988)), according to the current standard operating procedure (SOP). Antigen (Onderstepoort Biological Products (OBP), South Africa, batch 146) was added to test sera at room temperature ($22 \pm 4^{\circ}\text{C}$) and mixed in a WHO haemagglutination plate before incubation for four minutes on a rotary agglutinator (Heidolph polymax 2040, Heidolph, Germany). Any agglutination, as read immediately after incubation with the aid of an X-ray viewing box, was interpreted as a positive result.

COMPLEMENT FIXATION TEST (CFT)

The CFT was carried out according to the procedure of Alton and others (1988) by way of the warm fixation method, and according to the current SOP at OVI. The test was carried out in 96-well u-bottomed microtitre plates (NUNC, Thermo Scientific). The positive control serum was supplied by OBP (lot 5), while complement (batch 303 284) and amboceptor (batch 302 183) were supplied by Siemens (Germany). When the procedure was completed, the plates were read over a magnifying mirror by comparing the haemolysis to standards corresponding to 0-4 (where 0= 100% lysis, and 4= 0% lysis), and scored according to the International CFT Units/ml table. Only results above 20 ICFTU were considered positive.

INDIRECT ELISA (IELISA)

The iELISA assay was carried out according the manufacturer's instructions using the Chekit™ (batch BAT 1132 220 – X101, Idexx Laboratories, Switzerland). The plate was read at 450 nm in a microtitre plate reader (Powerwave X52, BioTek, USA) with the aid of Gen 51.11 software (BioTek). The results were analysed using the formula: Sample

OD% = (OD sample – OD negative control)*100/ (OD positive control – OD negative control). Test sera with optical density values equal to, or greater than 82% were considered positive.

RESULTS

NEGATIVE HERDS

All the 608 negative control animals tested negative to CFT and ELISA, with one animal testing positive to RBT. The average skin reaction to the BST was -0.11 mm (95% confidence interval -0.219 mm to -0.003 mm). There were neither visible nor palpable allergic reactions in this group.

INFECTED HERDS

Among the 846 animals in this group, 53 reacted positive on RBT, 58 were positive on CFT, while 126 were positive on iELISA. When animals reacting positive to at least two serological tests were taken as truly infected (Jacobson 1998), 63 such animals were identified in this group. In this group, the average reaction to the BST was 1.45 mm (95% confidence interval of 0.611 mm to 2.281 mm).

OPTIMISATION OF THE BST CUT-OFF VALUE

Estimation of the optimum cut-off value was done by receiver-operator characteristic (ROC) analysis using StatsDirect version 2,7,8 software.

The area under the ROC curve obtained was 0.763104, while the selected cut-off value was 0.7 mm. This resulted in sensitivity (capacity of test to give positive result in infected animals) of 53.97% (95% confidence interval: 40.94% - 66.61%), and specificity (capacity of test to give negative result in non-exposed animals) of 95.89% (95% confidence interval: 94.00% - 97.33%).

It was, however, found that shifting the cut-off point to 1 mm and incorporating all visible and or palpable allergic reactions as positive (Plommet (1984)) did not adversely affect sensitivity. Moreover specificity was improved to 99.01% (95% confidence interval: 98.22% - 99.80%). The results obtaining from negative control as well as truly infected animals are summarised in the 2 X 2 contingency table below.

Table 1: BST results in truly brucellosis infected as well as brucellosis free animals

BST		Brucellosis infected	Brucellosis free	Total
	Positive	34	6	40
	Negative	29	602	631
	Total	63	608	671

COMPARISON OF THE BST TO SEROLOGY IN INFECTED HERDS

The results obtaining from the performance of the BST against RBT, CFT and iELISA in 846 animals from brucellosis-positive herds are summarised in table 2 below

Table 2: Performance of BST against serology in 846 brucellosis exposed animals

	BST	RBT	CFT	iELISA	RBT & CFT	RBT & iELISA	CFT & iELISA	RBT, CFT, iELISA (any two in series)
Positive reactors	107	53	58	126	44	49	54	63
Reactor rate (%)	12.65	6.26	6.86	14.89	5.20	5.79	6.38	7.45

Table 3: Agreement between BST and serological tests in brucellosis-positive herds

BST	RBT		CFT		iELISA		RBT, CFT, iELISA (any two in series)		BST Total
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	
Positive	29	78	28	79	63	44	34	73	107
Negative	24	715	30	709	63	676	29	710	739
Total	53	793	58	788	126	720	63	783	846
Kappa coefficient	0.304		0.275		0.468		0.338		

In brucellosis free herds, the kappa coefficient of agreement between serology and the BST was zero.

DISCUSSION

The results of our study give supporting evidence that there is value in considering the BST as an additional test in the surveillance for brucellosis in unvaccinated animals.

The mean of the BST reaction in the 63 identified truly infected animals of 1.45 mm (95% CI: 0.61 -2.28) was consistent with earlier reports that positive brucellin response was less intense when compared with that of a tuberculin-specific reaction (Saegerman *et al* 1999) and that a cut-off value of 2 mm suggested by earlier researchers (Bercovich & Muskens 1999) adversely affects sensitivity of the test. There were no visible or palpable reactions in the brucellosis free herds and more than 90% of the animals tested had reactions less than 0.5 mm.

Although ROC analysis produced a cut-off value of 0.7 mm, it was felt to increase this value to 1mm and include all visible/palpable skin reactions as positive. The later interpretation of a positive reactor had no adverse effect on sensitivity, which remained at 53.97%, but improved specificity from 95.89% to 99.01%. The inclusion of all visible/palpable reactions as positive is also supported by earlier workers (Bercovich *et al* (1992), Saegerman *et al* (1999)). The BST sensitivity of 53.97% compares well to that of between 52% and 68% found by others (MacDiarmid & Hellstrom (1987), Pouillot *et al* (1997)), but lower than 81%, found by Bercovich and ter Laak (1990). The later study used bacterial isolation to define a truly infected individual in calculation of sensitivity. While this is the undisputed gold standard method, it is known to exaggerate the sensitivity of the assay under investigation (Jacobson (1998)). Saegerman and others (1999) found BST sensitivity of between 93% and 78% in experimentally infected animals at 27 and 187days post infection respectively. They found BST sensitivity decreased with increasing post-infection period. The BST sensitivity obtained in this study was however higher than that of 33% obtained by another study (Martrenchar, Njanpop, Yaya, Njoya, & Tulasne, (1993)).

The BST detected all brucellosis infected herds in the study as positive. The BST (12.65%) and iELISA (14.89%) had comparatively higher reactor rates in the infected herds, compared to RBT (6.26%) and CFT (6.86%). Although irrefutable evidence vis a vis the status the animals which tested positive on BST but negative on serology could only be obtained through slaughter and bacterial isolation, it was beyond the budget of this study. These findings are consistent with those of others (Kolar (1984), Fensterbank (1977)) who found the BST to detect infection early (before circulating antibodies), as well as in the chronic latent stages when serologic tests are often negative. Agreement between diagnostic tests, as measured by the kappa coefficient, was only fair between BST on one hand, and RBT (0.304) and CFT (0.275) on the other. This may be explained by the fact that they measure different analytes, cell mediated immunity for BST and antibodies for serology. The kappa statistic between BST and iELISA was 0.468. That iELISA is reputed to detect early brucellosis infections (Saegerman *et al* (2004)) may partly explain the better agreement between BST and iELISA. The study also noted that 29 animals were sero-positive, but negative on BST. Of these, 11 animals had CFT reactions above 344 ICFTU (six of these were 784 ICFU). This may be due to anergy as suggested by other studies (Cunningham, Miler, Dolan, McKeon & O'meara (1980)). Of the remainder, six had CFT titres of 60 ICFTU and below. It is possible the later may be cross-reactions to closely-related bacteria.

The foregoing argument supports the recommendation to include the BST in the South African brucellosis diagnostic test panel, especially when used in parallel with serology. Used this way, the potential to detect acutely as well as chronic latently infected unvaccinated animals is improved, along with a reduction in the time it takes to clear a herd of infection as infected animals may be identified before parturition and therefore averts potential spread.

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AN INVESTIGATION INTO THE CAUSE OF HIGH ABORTION RATES OF GOATS IN THE LIMPOPO PROVINCE, SOUTH AFRICA.

Ndou, R.V. and Dlamini, M.L.,

The study was carried out to investigate the cause of exceedingly high abortion rates and other reproductive failures of goats at Vhembe district, in the Limpopo Province, South Africa. Reproductive failures are of great economic importance to goat farmers and can result from various factors that include infectious agents, nutritional deficiencies and environmental factors. Additionally, goat abortion due to an infectious agent is a public health risk since most abortifacient agents are zoonotic. *Toxoplasma gondii* (*T. gondii*), *Chlamydia abortus* (*C. abortus*) and trace mineral deficiencies were investigated as possible etiologies in this situation. A total of 338 blood samples were collected from 22 herds and enzyme-linked immunosorbent assay (ELISA) was used to determine the seroprevalence of *T. gondii* and *C. abortus*. Copper and Zinc serum levels were analyzed using the Atomic Absorption Spectrophotometer (AAS) machine and Selenium was analyzed using the ICPMS 300Q machine. During sample collections, interviews were also conducted using a questionnaire to gather farm management information and to assess food safety and zoonoses knowledge amongst farmers. Seroprevalence of *T. gondii* in herds ranged from was 0 to 38.5% ($\mu = 9.2\%$). An overall prevalence of 30.4% to *C. abortus* antibodies was recorded with a 100% seroprevalence detected in one herd. Zinc and Selenium deficiencies of up to 12.5 and 5000 times respectively, lower than the minimum expected levels were revealed in all areas, whilst copper levels exceeded the maximum expected by up to 4.4 times. Interviews exposed a lack of awareness about *T. gondii*, *C. abortus* and other public health issues; furthermore they also highlighted the lack of supplementary feeding and basic herd health management practices amongst farmers. In this study *T. gondii*, *C. abortus* and trace mineral deficiency were indicated as significant contributors to the abortion in the study areas.

INTRODUCTION

Abortions and other reproductive failures in small ruminants are of great economic importance to farmers worldwide (Abd El-Razik *et al.*, 2011), however a 2-5% abortion rate in a herd is expected, and acceptable (Schoenian, 2000). There are various factors that contribute to reproduction failures in goats and those include infectious agents, nutritional deficiencies, environmental and seasonal factors. Goats' reproductive cycles are influenced by photoperiod, temperature, rainfall cycles, pheromones and social cues (Arroyo *et al.*, 2000).

In goats, abortions due to infectious diseases are a public health concern as most of the abortifacient pathogenic agents are zoonotic and in Africa, women and children are at a greater risk as they are frequently involved in smallstock husbandry (Yilmaz *et al.*, 2002). The agents frequently incriminated in abortion cases include *Brucella melitensis* (*B. melitensis*), *Toxoplasma gondii* (*T. gondii*), *Chlamydia abortus* (*C. abortus*), *Listeria monocytogenes*, *Coxiella burnetii* (Q fever), *Salmonella* spp and *Campylobacter fetus* (Longbottom *et al.*, 2003; Darwish *et al.*, 2001). However, nutritional deficiencies also play a major role in the reproductive failures in goats. Zn, Copper, and Selenium are the trace minerals that successfully govern the reproductive behaviour and processes in small stock (Wildie, 2006) and their availability depends on forages and feed quality (Vazquez- Armijo *et al.*, 2011).

Studies in different countries have revealed prevalence of *T. gondii*, *Chlamydia abortus*, and trace mineral deficiencies as amongst the top causes of abortions in goats. In a study by Robert and Moeller 2001 in the Philistines, 37% of caprine abortions were associated with infectious agents, whereby bacterial agents accounted for 30.5%, viral for 2%, fungal for 0.5%, and protozoal (*T. gondii*) for 4%. *Chlamydia abortus* and *Coxiella burnetii* were the highest isolated infectious agents at 23%. In the same study, mineral deficiencies (4%) especially in Copper and Selenium were also reported. In Botswana a seroprevalence study for the antibodies to *T. gondii* and *C. abortus* was performed in goats with a history of abortion, stillbirth, and neonatal mortality resulted in those agents being implicated (Sharma *et al.*, 2003).

In South Africa limited studies have been done focused on causes of smallstock abortions, Ndou *et al.*, 2011, found 4.3% seroprevalence of *T. gondii* in goats in the Molopo district, North West Province, while Abu Samra *et al.*, 2007 found an overall seroprevalence of 5.6 % using (IFA) and of 4.3 % using (ELISA) in sheep in KwaZulu-Natal, Eastern Cape, Western Cape, Gauteng Provinces. Those studies were focused on the public health aspect of the disease but also give a picture of what is happening in the country. A serosurvey of *T. gondii* infection in HIV patient at Helen Joseph Hospital, Johannesburg, South Africa, detected using ELISA a 25(8%) prevalence of *T. gondii* antibodies, whilst 22 of those patients had a reactivation toxoplasmosis, 0.68% with clinical manifestation of Toxoplasma Encephalitis (TE) and retinitis (Hari *et al.*, 2007). Pascal *et al.*, 2010 reported 18.1% seroprevalence of *T. gondii* in cross- sectional study of HTLV1/2, HSV1/2 and *T. gondii* from of HIV infected individuals attending health establishments in Musina, Bela Bela and Madibo in the rural Northeastern South Africa. In 2006, the National Institute Communicable Disease of South Africa expressed a great concern about the lack of data on the prevalence of

T. gondii in the South African public; therefore, they have undertaken studies to collect data. As of now there are no published reports on the seroprevalence of *C.abortus* in small stock and human in South Africa.

In African countries, goats are very important both economically and socially. Apart from being a status symbol in some indigenous communities, they are a ready source of income and meat. In South Africa, small ruminants including goats are an important source of protein for humans. Furthermore, goats play an important role in the socio-cultural lives of communities where they serve during rituals (Ndou *et al.*, 2011).

Frequent abortions in goats in the Mutale and Makhado municipalities of Vhembe district, Limpopo Province are an economic burden to farmers. During the last three years, goats have been aborting especially around winter and this has resulted in some farmers having adult goat only herds, as all pregnancies had resulted in abortions, stillbirth, or weak kids that subsequently die. Abortion in goats is also of a public health issues as most of the abortifacient infection agents are zoonoses that can be fatal to humans. High prevalence of abortions in goats therefore needs to be investigated so that farmers may implement informed management and prophylactic measures.

The objectives of the study were to determine the serologically determine the cause of abortions in Mutale and Makhado State Veterinary areas Limpopo Province. Also assess risk for potential public health hazards as a result of exposure to abortifacient pathogens.

MATERIALS AND METHODS

STUDY AREA

Samples were obtained from the rural areas of Vhembe district, Limpopo Province, South Africa from the 28th – June to 1st July 2011 at Makhado and Mutale Municipalities. Makhado Municipality is situated at the foot of the densely forested Soutpansberg Mountain Range, near the Zimbabwean border and is a rapidly growing agricultural area with a climate that varies from Savannah plains, bushveld to sub-tropical. Its jurisdiction is divided into four distinct regions, namely Vuwani, Dzanani, Waterval and Makhado. Mutale municipality is situated in the far north-eastern part of the Limpopo Province, bordering the Republic of Zimbabwe in the North and the Republic of Mozambique in the East through the Kruger National Park. Drought conditions are common in most parts and its biomes is Savannah with most of the region been classified as rural with small-scale agriculture and subsistence farming.

For the purpose of this study the study areas were classified as areas 1-4. Area 1 which is in the Mutale municipality included Tshiungani and Tshidzi villages (22° 31' 34.7" S, 30° 37' 11" E), Area 2, Tshimboni village (22° 31' 19" S, 30° 35' 42" E) in Mutale municipality. Makhado municipality included Bungeni (23° 12' 12.2" S, 30° 16' 20.8" E) classified as (Area 3) and (Area 4). Area 4 Mara (Goedegedacht farm) (25° 4' 26" S, 29° 35' 44" E).

BACKGROUND INFORMATION OF THE STUDY AREA

The study was undertaken as a response to the continual reports of high levels of abortions in goat herds, in Makhado and Mutale municipality with various farmers experiencing high abortion incidences for the past three years. The survey focused on most of the possible causes of abortion including *Brucella melitensis* (*B.melitensis*), Rift Valley Fever(RVF), Wesselbron disease (WD), *Toxoplasma gondii* (*T.gondii*), *Chlamydomphila abortus* (*C.abortus*) and nutrition factors (Zn, Se and Cu as indicators of deficiencies), however, only the findings for *T. gondii*, *C.abortus* and trace mineral status will be discussed in this manuscript.

MAP OF STUDY AREAS

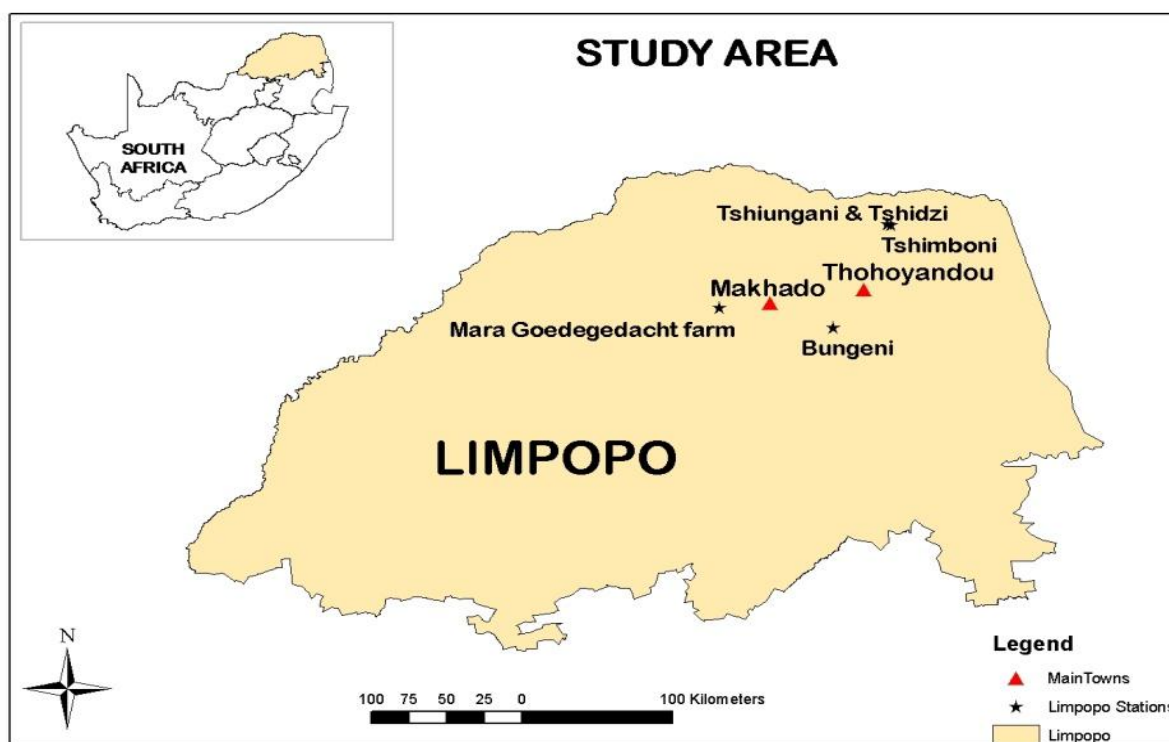


Figure 5: On the map Limpopo stations refers to the study areas 1-4

SAMPLING METHOD AND SAMPLE SIZE

Twenty-two herds were randomly selected from those farms that had a history of abortions and those of their neighbours without abortions. A total of 338 goat's blood was collected.

Blood samples were collected into serology tubes from the jugular vein and transported to the Sibasa State Veterinary Laboratory. The blood was left at room temperature overnight to allow clotting. The sera were separated after centrifugation at 2500rpm for 10 minutes and stored in serum tube then transported to the North West University Hematology lab on ice where they were stored at 20 °C until analysis.

SEROLOGICAL ANALYSIS/TESTING

TOXOPLASMA GONDII

The checkit*tox ELISA test kits (LOT number 192- T941) were obtained from IDEXX Switzerland AG with full instructions and used to test for T.gondii antibodies in the sera. The kit test provides rapid, simple, and specific method for detecting antibodies against Toxoplasma gondii in serum and plasma of small ruminant.

Plate reading

The ELISA machine (ELISA Multiscan ex RS-232C) was used to read the plate at a wavelength of 450nm to obtain the optical density.

Calculation of the optical density

The OD value of duplicates must be average of the positive control (OD_{pos}) and the OD_{sample}) are corrected by subtracting the OD OF the negative control (OD_{neg}) Analyze the sample in the relation to the negative and the positive controls with the formula:

$$Value\% = \frac{OD_{Sample} - OD_{neg}}{OD_{Pos} - OD_{Neg}} \times 100\%$$

Interpretation of the results

Sera were negative if the value < 20% and it becomes suspect $\geq 20\%$ to < 30%. Test sera were considered weak positives if the value is $\geq 30\%$ to < 100% and it is positive if the value is $\geq 100\%$, as illustrated below.

Value	< 20%	$\geq 20\%$ to < 30%	$\geq 30\%$ to < 100%	$\geq 100\%$
Interpretation	Negative	Suspect	Weak positive	Positive

CHLAMYDIA

A total of 23 serum samples were selected from some of the aborted does and sent to the ARC- Onderstepoort Veterinary Institute Molecular Epidemiology and Diagnostics laboratory through the Sibasa State Veterinary office for the I-ELISA test to determine the presence of *C.abortus* antibodies in the serum.

MINERAL ANALYSIS

PREPARATION AND ANALYSIS

Zinc (Zn) and Copper (Cu)

Serum was diluted into distilled water (1ml serum into 5mls of distilled water) for the analysis of Zinc (Zn) and Copper (Cu) and were analyzed using the Atomic Absorption Spectrophotometer (AAS) machine, ICP MS using approved methods from the Perkin Elmer release Version E (2000).

Selenium (Se)

10g of Tetra Acetic Chlorine Acid (TCA) was dilute into 100mls of distilled water in a 100 ml volumetric flask.), 0.7mls of serum was precipitated into 7mls of TCA in a test tube and centrifuge at 2500 for 10minutes. 5mls of supernatant was transferred into a sterile test tube and analyzed using the ICPMS 300Q machine.

Results Interpretation

The recommended upper and lower critical limits for serum concentration of trace minerals in goats will be used to assess the trace mineral content in the sera (Table 1).

Table 2: The normal serum concentration range values for Cu, Zn and Se in goats. (Fujihara et al., 1992; Orden et al., 1999; Shinde and Sankhyan 2007)

Elements	Lower limits	Upper limits
Copper (Cu)	0.6(0.58mg/l)	1.60mg/l
Zinc (Zn)	0.6mg/l	1.60mg/l
Selenium (Se)	20ug/l	200ug/l

Statistical analysis

The SPSS software was used for analysis to compare the means and state any significance ($p \geq 0.05$) variation amongst each and element in the various areas. Comparison by Duncan's multiple range tests was used to detect statistical significance between the areas.

QUESTIONNAIRES AND OBSERVATIONS

A questionnaire was designed with three sections, section 1 was demographics including the education level of each respondent, section 2 focused on herd health management, feeding practices and abortions in the herd and section 3 was on food safety and zoonoses knowledge. After blood collection interviews were conducted with either a farmer or the herder using questionnaires (attached as Annexes A).

Observations were made in each herd to evaluated farm location, farming system, presence of stray cat, wild felines and domestic cat, rivers and other factors that could be a source of *T.gondi*

RESULTS

T. GONDII

A total number of 338-goat sera were obtained from 22 goat herds in Mutale and Makhado municipalities, Limpopo Province. In Mutale samples were collected at Tshiungani and Tshidzi villages (Area 1) and Tshimboni village (Area 2), while areas from Makhado included Bungeni (Area 3) and Mara (Area 4). The overall prevalence in the study was

9.2% with Makhado municipality recording the highest seroprevalence of 14.9% and Mutale the lowest at 7.7% (Table 2).

In all herds in the two municipality, the highest seroprevalence was in Area 4 (38.5%) at Mr Ndou's herd and lowest at Ms Malindi's herd at 7.7%. Fifteen herds out the total of 22 were seronegative. In the two herds in Area 2 (Mr Mavhusha and Mr Neludane's herds) antibodies against *T. gondii* were not detected. In Mutale municipality, Mr Makhado's herd had the highest prevalence at 26%, followed by Mr Nemaconde at 21.4% and Ms Ravhuanzwi and Mr Manari at 14.3%. The overall prevalence at Area 1 was 9.5% and 33.3% of those herds were seropositive. Area 3, which is the Mutsila goat project, was also seronegative for the antibodies against *T. gondii*. The study also observed that all 45 males sampled were seronegativity therefore with all seropositive results from the 293 females.

Table 3: *T. gondii* seroprevalence results in Mutale and Makhado state veterinary districts.

Sampling Areas	Location of herds	Farmers' Name	Total No of Sampled Animals/herd	No Seropositives	of Percetange (%) of Seropositive
Area1	Tshuingani	Makhado	50	13	26.0
	Tshidzi	Redali	5	0	0.0
		Lidovho	5	0	0.0
		Musanda	7	0	0.0
		Ravhuanzwi	7	1	14.3
		Nemakonde	14	3	21.4
		Manari	7	1	14.3
		Malindi	26	2	7.7
		Nedohe	11	1	9.1
		Mukwevho	27	0	0.0
		Colbert	6	0	0.0
		Thivhase	8	0	0.0
		Thenga	7	0	0.0
		Mudau	11	0	0.0
		Mutengo	7	0	0.0
		Tshililo	6	0	0.0
		Ragimani	6	0	0.0
		Ligunuba	10	0	0.0
Total			220	21	9.5
Area 2	Tshimboni	Mavhusha	31	0	0.0
		Nelundani	20	0	0.0
Total			51	0	0.0
Total Municipality			271	21	7.7
Area 3	Bungeni	Mutsila projects	41	0	0.0
Area 4	Mara	Ndou	26	10	38.5
Total Municipality			67	10	14.9
Overall Total			338	31	9.2

CHLAMYDOPHILA ABORTUS

Twenty-three samples from aborted does were selected and tested for the antibodies against *C.abortus*. Seven out of the 23 sera 30.4% were positive for *C.abortus*. Ravhuanzwi recorded a 100% seropositive to *C.abortus*. The overall prevalence was 30.4% (Table 3).

Table 4: Seroprevalence of *C.abortus* using I – ELISA

Famer's Name	No of sampled Animals	Status of Abortion	No of Seropositive	% Seropositive
Makhado	7	Aborted	0	0
Ravhaanzwi	7	Aborted	7	100
Mavhusha	3	Aborted	0	0
Nelundani	6	Aborted	0	0
Total	23		7	30.4

MINERAL RESULTS

Sera were tested for Cu, Se, and Zn, in Mutale and Makhado municipalities and results are shown in figures 1 and 2 below. Area 1 had the highest concentrations of Cu, Zn and Se while area 4 generally had the lowest concentrations (fig 1 and 2). Lowest levels of Se were recorded in area 3. Mean Zn, Se and Cu concentrations ranged from 0.0478 - 0.1493 mg/l, 0.00408 – 0.0044 ug/l, and 6.9815 - 7.0873 mg/l respectively. Significant differences in mineral concentrations were noted among some areas (Table 5).

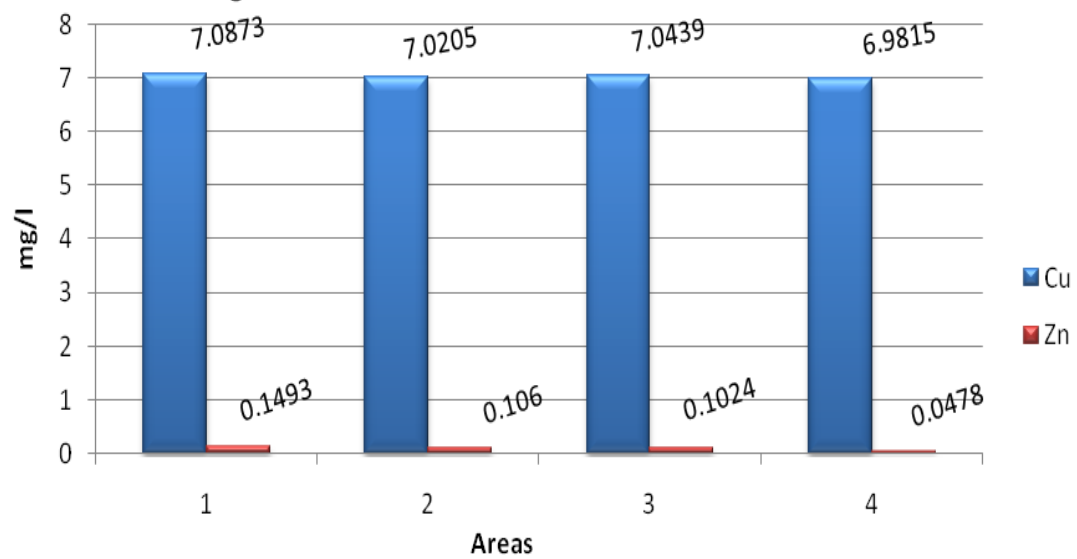
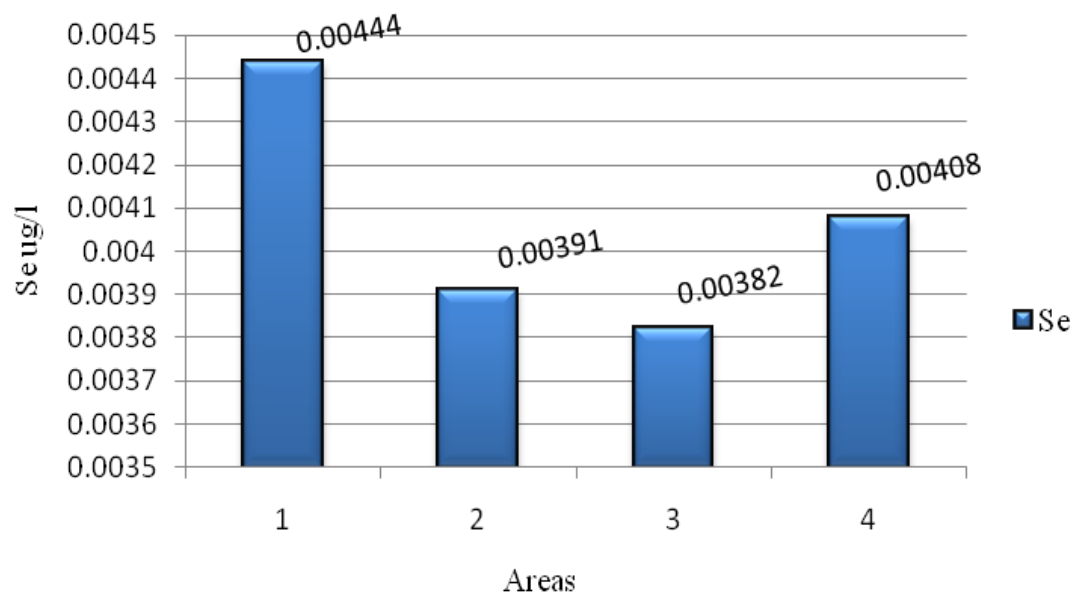
Fig1: Cu and Zn blood mean concentration**Fig2: Se blood mean concentration**

Table 5: Mean concentrations of Cu, Zn and Se in goat sera. a,b,c: figures with similar letters were not significantly different.

Elements	Areas	Mean
Copper (mg/l)	1	7.0873 \pm 0.29 ^b
	2	7.0205 \pm 0.14 ^b
	3	7.0439 \pm 0.23 ^b
	4	6.9815 \pm 0.21 ^a
Zinc (mg/l)	1	0.1493 \pm 0.03 ^c
	2	0.1060 \pm 0.02 ^b
	3	0.1024 \pm 0.01 ^b
	4	0.0478 \pm 0.01 ^a
Selenium (ug/l)	1	0.00444 \pm 0.00531 ^b
	2	0.00391 \pm 0.00039 ^a
	3	0.00382 \pm 0.00025 ^a
	4	0.00408 \pm 0.00012 ^b

QUESTIONNAIRES AND OBSERVATIONS

A total of 22 individuals were interviewed using questionnaires. The respondents included 5 farmer and 17 goat herders. The level of education for all the farmers was tertiary education whilst all herders had education level less than grade 7. The purpose of farming for 19 of the farmers was subsistence with one commercial farmer and two emerging farmers. The abortion rates in each herd were above 20%, whilst Ms Ravhuanzwi had the highest of 100% abortion.

Only one farmer Mr Neludane vaccinated and dewormed his herd for various diseases that does not include *C.abortus*. Seven farmer provided supplementary feed for their goats, Mutengo, Ragamani, Madau, Thenga, Radali, Neludane, Mutsila project and Ndou supplemented the goats with lucerne, pellets and hay but they all still had abortion. In Tshidzi and Tshimboni 10 of the respondent denied the presents of domestic and wild cats the area whilst 11 were not certain, however Mukhwevho had domestic cats in his household, the Goedgedacht farm is located next to a game farm, which makes the presence of wild cats a certainty. 80% of the respondent revealed that aborted fetus though handled by naked hand were buried whilst 20% of them (all herders) said they left the fetus in the kraals until their employers have seen them.

Total lack of knowledge of some zoonotic diseases was discovered in all the respondents with 50% of them consuming goats' raw milk. All food safety questions revealed a 100% ignorance level for all answers whilst 90% of the farmers practised informal slaughters.

Observations revealed that the sampled areas were located in rural settlement and the areas in Mutale were very dry and with hardly anything for the goats browse except bitter leaf shrubs. Tshiungani, Tshidzi, and Tshimboni had barest grazing land, whilst Goedgedacht farm in Mara had more than sufficient forage for the goats. The climatic conditions in Tshiungani, Tshidzi, and Tshimboni favours only goats farming and herd size ranged from 5 to 50 goats. In this population, Boer goats dominated with few cross breed and indigenous breeds. It was also noted that newborn kids and does had poor body condition scores.

Boreholes are the main source of drinking water Mutale areas however rivers are available though distant (5km) for the goats to drink. In Makhado, Mutsila project provided water from drinking troughs obtained from boreholes and Goedgedacht farm had borehole and a small river running through the farm.

DISCUSSION

To our knowledge this is the first study of prevalence to antibodies against *Toxoplasma gondii* and *Chlamydophila abortus* infection and trace mineral deficiencies assessment in Vhembe district and their contribution to the abortions in that area. The investigation was prompted by the continuous reports of abortions in goat herds at Mutale and Makhado municipalities. Abortion rate in goats is acceptable when it is between 2-5%, an increase above this warrant an investigation to determine the cause (Van Saun, 2006). The farmers in the study area had experienced abortions for

the last three years with some getting as high as 100% abortion rate. Worldwide the frequently incriminated abortifacient infectious agents in goat's cases are *Brucella melitensis*, *Toxoplasma gondii*, *Chlamydophila abortus*, *Listeria monocytogenes*, *Coxiella*, *Salmonella* and *Campylobacter fetus* (Longbottom *et al.*, 2003; Darwish *et al.*, 2001). Other factors that induce abortion include excessive exercise, nutritional deficiencies and environmental conditions. During the study all of the above mentioned were considered and investigated but for the purpose of this manuscript only *Toxoplasma gondii* and *Chlamydophila abortus* infection and trace mineral deficiency findings will be discussed. Small ruminants, including goats are an important source of protein for humans. Furthermore, goats play an important role in the socio-cultural lives of communities in rural South Africa where they serve during rituals (Ndou *et al.*, 2011), they are also a status symbol in most of these communities. Additionally goat farming is common in the communal pasture due to cost effectiveness of goat rearing. According to the Goats MVCP commodity statistics profile of 2009-2010, Limpopo province had about 24% population of goats in communal pastures. During data collection it was observed that goat farming dominated the Mutale and Makhado municipalities, out of a total of 22 study herds only 3 farmers' also farmed sheep and cattle. This is due to the climate and vegetation especially in Mutale municipality which is very dry and prone to drought conditions as caprines are good browsers.

Abortions due to infectious diseases also pose a great public health risk as some of those pathogens are zoonotic and in most cases will also lead to illness in humans. In Africa, the population at higher risk is women and children that closely involved in the farming of goats (Yilmaz *et al.*, 2002). Additionally, in most rural settings goats are slaughtered informally with no meat inspections conducted especially during traditional ceremonies and if cultural consumption of raw meat and eating of undercooked braai meat is practiced, there is a great possibility for people to be infected. Furthermore, herd health management practices are poor in communal areas and the eating of diseased animals is quite common thereby increasing the risk for people acquiring zoonotic diseases and food borne infections.

TOXOPLASMA GONDII

T. gondii is one of the frequently incriminated pathogens in abortion cases in small stock (Longbottom *et al.*, 2003); it causes abortions by infecting the placenta during the tachyzoites stage of the life cycle, thereafter passing to the fetus, causing fetal death, fetal mummification, stillbirth, or the birth of weak kids. The abortions usually occur in the last trimester of pregnancy and may occur in does of all ages and in successive pregnancies. (Buxton, 2007). Cats are the definite host of this protozoan and pass the oocyst in their faeces, thereby contaminating the environment (Dubey, 1998). *T. gondii* is also an important zoonotic disease that can cause severe illness and conditions in humans. Infections of pregnant women may lead to abortions and neonatal toxoplasmosis. It has been recognized as one of the most important opportunistic infections for immune-compromised individuals, this has led to it being included in agendas of most HIV and AIDS discussions. The disease in humans causes retinitis and highly fatal toxoplasma encephalitis, in Helen Joseph Hospital, Johannesburg, it was serologically detected in 25(8%) HIV patients (Hari *et al.*, 2007). A seroprevalence of 18.1% of *T. gondii* was reported in a cross-sectional study of HTLV1/2, HSV1/2 and *T. gondii* from HIV infected individuals attending health establishments in Musina, Bela Bela and Madibo in the rural Northeastern South Africa (Pascal *et al.*, 2010). Considering the seriousness of the situation with HIV and AIDS in our country, the lack of data and historical information of the disease is of great concern, this has led the National Institute Communicable Disease of South Africa in 2006 to declare their intention to remedy the situation by undertaking researches in *T. gondii*.

SEROPREVALENCE IN MAKHADO AND MUTALE MUNICIPALITIES

The study revealed an overall seroprevalence of 9.2% in Vhembe district with Mutale and Makhado municipality at 7.7% and 14.9% respectively. These findings are higher than the 4.3% detected in goats in Molopo district (Ndou *et al.*, 2011) and 6.4% in sheep five Provinces in South Africa (Abu Samra *et al.*, 2007). The lower prevalence in Mutale could be attributed to the dry climatic conditions of the district. Makhado municipality also had the highest herd seroprevalence at the Goedegadacht farm of 38.5% and this was attributed to the presence of wild cats as the farm is located next to game farms and the wetter climatic conditions of the area.

The overall prevalence is low compared to the studies done in neighbouring countries, in Zimbabwe 67.2% was reported in adults sheep and goats (Hove *et al.*, 2005) and in Botswana the prevalence was 10% which is closer to this study (Binta *et al.*, 1998). Higher prevalence were expected because Limpopo is a wet area (warm moist humid climate) as *T. gondii* favours warm moist areas for its survival in soil, but Mutale lies in the area of Limpopo which has very dry conditions especially in winter. Other factors play a role in the prevalence of *T. gondii* those include, presence of felines, rivers and stream (Abu Samra *et al.*, 2007). In Mutale municipality, animals had no access to rivers and the main source of water was boreholes, whereas in Goedegadacht farm has a small river running through the farm that animals utilize.

SEROPREVALENCE AND MANAGEMENT SYSTEMS

Goats raised under extensive management present with higher prevalence due to extensive exposure in the environment. In this study the highest prevalence of 38.5% was obtained in Goedegeacht farm a herd under extensive management system. According to Ghazaei, 2010, goats bred and raised under extensive management run higher risk of exposure to *T. gondii* oocysts from pasture and water as compared to goats under intensive farming systems.

SEROPREVALENCE AND GENDER

The study comprised of 293 females and 45 males and only female goats tested seropositive thereby establishing a correlation between sex and rates of infection. Females are more susceptible to *T.gondii* infection than males, due to sex hormonal changes, physiology and handling differences (Silva et al., 2003 Vander Puije et al., 2000). Martin 2000, Kelly et al., 2001, Uzeda et al., 2004 added that higher infection rate in females could be attributed to possible immunosuppression related to nutrition, gestation and lactation events. While Teshale et al., 2007 stated that females are kept longer for breeding and milking production than males therefore increasing the possibility of exposure.

CHLAMYDOPHILA ABORTUS

C.abortus is common cause of abortion worldwide, and in some countries where *Brucella melintisis* is under control, it is the most frequent cause of abortion in goats. It is also associated with pneumonia, pink eye, inflammation of epididymis, and inflammation of the joints. The bacteria cause abortions in does by evoking a placentitis with abortions occurring any time between days 100 and 130 of gestation Rodolakis et al., 1998.

C.abortus is a serious zoonotic disease associated with abortion cases and severe illness as a result of exposure and contact with placentas of *C .abortus* infected does (Ward 2006). Positive correlations in many studies have been established that links abortions in does linked to abortions in the farmers' family (Yilmaz, 2002). Studies and published work on the disease in South Africa were not found during literature review.

SEROPREVALENCE OF C.ABORTUS

A total of 23 samples from does that had aborted were analyzed from four herds in Mutale municipality, the study revealed an overall seroprevalence 30.4% *Chlamydomphila abortus*. This overall prevalence was high compared to studies in other countries; in Namibia, 8% seropositives was obtained for individual animals (Samkange et al., 2010) and in Saudi Arabia 9.07% was obtained from aborting does (Abd- El-Razik et al., 2011). In the Philippines *C.abortus* was established as the main cause of abortion by 14.2% prevalence (Robert and Moeller, 2001). In addition in this study, a farmer had all her female goats aborting and had 100% prevalence of *C.abortus* and all other herds were seronegative, it is to be noted that the same farm had a 14.3% *T.gondii* serological prevalence. This does not establish any correlation between the two pathogens due to the difference in sample sizes but indicates that in farms where both agents are present the abortion rates would very high. Also it demonstrates that in herds where *C.abortus* is present and not treated a 100% mobility rate of the disease is possible. Besides being public health risk, is also an economical problem to farmers due to the abortions.

TRACE MINERALS

Mineral concentrations obtained from this study indicated significant mineral deficiencies in Zn and Se in all the four areas. Zn and Se were lower than their expected lowest limits of 0.6mg/l and 20ug/l by up to 12.5 and a massive 5236 times respectively. Trace minerals have been shown in numerous studies to be essential to maintain high fertility in ruminants; they are needed for vitamin synthesis, hormone production, and other physiological processes related to growth, reproduction, and health. Zinc, Copper, and Selenium are the minerals that successfully govern the reproductive behaviour and processes in small stock (Wildie, 2006). Se deficiencies have been associated with lamb mortality, reduced sperm motility and uterine contraction, cystic ovaries, low fertility rate, retained fetal membranes while those of Zn have been associated with impaired spermatogenesis and development of secondary sex organs in males and reduced fertility (Vazquez-Armijo et al., 2011). Also, low conception rates, abnormal oestrus behaviour, infertility, abortion, and neonatal mortality can be associated with trace elements deficiencies (Van Metre and Callan, 2001). In this study, all areas revealed deficiencies in Zn and Se, and also recorded incidences of abortions (Table 5). However, the observation that area 1 was also seropositive for *C.abortus* (Table 4) and areas 1 and 4 also seropositive for *T. gondii* (Table 3) is indicative of the huge possibilities of multi-causal relations with respect to reproductive problems in the areas. More studies would there be required to determine the specific etiologies.

Subclinical trace minerals deficiencies occur more frequently than can be recognized by most livestock owners. This may represent a more serious problem than clinical trace elements deficiency because owners and farmers do not

recognize specific signs that are characteristic of subclinical trace elements deficiency. Instead, the immune system is depressed, the animal grows more slowly, and fertility is impaired. The result is inefficient production and lowered fertility.

The deficiency in serum could be an indication of lower forage mineral status, which could also be related to the seasonal changes, climatic conditions and soil mineral quality (Orden *et al.*, 1999). The trace mineral deficiency in Zn and Se exhibited by goats from Mutale and Makhado could therefore have been due to insufficient minerals from plant feed stuff which could affect their reproductive performance. More studies are indicated to investigate the possible reproductive implications to these deficiencies in the studied herds.

In this study, mean copper Cu levels exceeded the expected upper limit of 1.6mg/l by up 4.4 times, thereby possibly exposing the goats to toxicity. Higher levels of Cu in blood may impair liver function, subsequently disturbing reproductive processes and behaviour in ruminants (Van Saun, 2006).

PUBLIC HEALTH RISK ASSESSMENT TO T.GONDII AND C.ABORTUS

The overall seroprevalence of *C.abortus* and *T.gondii* at 30.4% and 9.2% respectively in the study poses a great potential risk of infection to the human population, because goats are bred for meat production and eventually joins the food chain. The total lack of general knowledge on the epidemiology, control and prevention measure of these diseases revealed in the study could encompass significant public health risk. Previous epidemiological studies have stated that consumption of raw or undercooked goat meat contaminated with cysts, or unpasteurised goat milk (Pepin *et al.* 1997), and drinking water containing *T.gondii* tachyzoites coupled with poor hygiene and improper food handling practices increase the risk for human infection (CDC, 2000). In the current study 100% of the farmers had no knowledge about the pathogens and 50% consumed unpasteurised goats whilst 90% slaughtered their animals informally. While cooking practice are generally adequate to destroy the infectious in tissue cyst but consumption of roasted (undercooked meat) and traditional eating of raw meat could be a possible risk factor for human infection (Bisson *et al.*, 2000). Therefore, general hygiene and proper food safety handling (sanitary kitchen habits) measures must be emphasized, thorough cooking of meat, vigorous hand washing before meals or after doing outdoor activities such as gardening, having a healthy person dispose of cat litter boxes rather than pregnant women, the use of gloves in cat box disposal, and the pasteurization of milk before consumption by humans (Cook *et al.*, 2000, Lopez *et al.*, 2000). Continuous serological evaluation of food animal and screening of the human population is vital in determining the risk of human infections. Toxoplasmosis is one of the fatal opportunistic infection in humans infected with the AIDS virus, resulting in severe toxoplasmosis that causes damage to the brain, eyes, or other organs after an acute infection or reactivation of an earlier infection (Hill and Dubey, 2002). According DOH 2007 statistics, 68% of people in Sub Saharan African Region are affected by HIV – Aids pandemic. Limpopo province is one of the Provinces faced with challenges of the HIV- AIDS, a prevalence study by Ramathuba and Maselesele, 2011 revealed the prevalence in antenatal clinics at 20.8% and that of the general public at 8.3% in Thohoyandou.

C.abortus transmission to human requires direct and indirect contact with a sick or aborting animal, presence of the pathogen in herds have been linked with illness and abortion in human (Aitken, 2000). The 100% prevalence in a Mutale herd increase the risk of human infections through aborted fetuses as it was revealed proper disposal of carcasses is not being practiced in the areas.

CONCLUSION

The findings of this study established *T.gondii*, *C.abortus* and trace mineral as the causes of the abortions in Mutale and Makhado municipalities. The study revealed the necessity of further epidemiological studies of infectious diseases and mineral deficiency causing abortion in small stock under different climatic conditions in South Africa.

The mineral status is an indication for farmers in Mutale and Makhado municipality to initiate supplemental feeding especially in winter and this is more critical in Mutale where winters are drier. Additionally herd health management practices must improve and should include vaccinations and deworming.

Basic food safety knowledge extension to the public of South Africa is indicated to improve awareness levels of foodborne and zoonotic diseases and proper food handling techniques.

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EPIDEMIOLOGY AND MANAGEMENT OF A BOVINE BRUCELLOSIS CLUSTER IN NORTHERN IRELAND

D.A. Abernethy^{a,*}, J. Moscard-Costello^b, E. Dickson^b, R. Harwood^b, K. Burns^c, E. McKillop^c, S. McDowell^c, D.U. Pfeiffer^d

ABSTRACT

An epidemiological investigation was undertaken of 41 bovine brucellosis outbreaks that occurred within a 10-month period, in a region where eradication measures appeared to be succeeding. The primary outbreak comprised three herds with significant within-herd spread and a high probability of multiple abortions. Direct contact between cattle at pasture was the most likely means of between-herd transmission for most (71%) outbreaks, with an attack rate of 28.1% in herds immediately neighbouring the primary outbreak herds and 11.3% in the next concentric ring of farms. Resolution of the incident was attributed to a rapid response by the veterinary authorities, detailed epidemiological investigations, repeated, prolonged testing of contact herds and employment of parallel testing.

^a Veterinary Epidemiology Unit, Department of Agriculture and Rural Development, Room 921/3, Dundonald House, Newtownards Road, Belfast, Northern Ireland, BT4 3SB, United Kingdom

^b Armagh Divisional Veterinary Office, Department of Agriculture and Rural Development, Mall West, Armagh, Northern Ireland, BT61 9BL, United Kingdom

^c Veterinary Sciences Division, Agri-Food and Biosciences Institute, Stoney Road, Belfast, Northern Ireland, BT4 3SD, United Kingdom

^d Epidemiology Division, Royal Veterinary College, Hawkshead Lane, North Mymms, AL9 7TA, United Kingdom

ANTIGENIC AND GENETIC CHARACTERISATION OF DOG RABIES VIRUSES RECOVERED FROM OUTBREAKS IN SOUTH AFRICA (2005-2011).

Phahladira, B., Mohale, D., Miyen, J., Shumba, W. & Sabeta, C.*

ABSTRACT

Dog rabies is enzootic throughout Asia and Africa including the Republic of South Africa (RSA) particularly in the coastal KwaZulu Natal (KZN), Limpopo, Mpumalanga and Eastern Cape (EC) provinces. From January 2005 to December 2011, a total of 8143 brain tissues were submitted to the OIE Rabies Reference Laboratory from all over South Africa for testing for the rabies virus antigen. Of the total submissions, 2853 samples [35%] were positive for the lyssavirus antigen and almost half [46.6%] of all positive cases were of dog origin [1329 samples]. The positive rabies samples were further characterised using a panel of anti-nucleocapsid monoclonal antibodies and selected samples sequenced by targeting a partial region of the glycoprotein gene. Antigenic characterisation differentiated the viruses into two species namely rabies virus [99.5%] and Mokola virus [0.5%]. During the period under review and in three instances [2005, 2008 and 2010], dog rabies outbreaks were confirmed and their origins traced back to specific geographical localities using phylogenetic reconstruction and mapping to the KZN province and the southern region of Zimbabwe respectively. The third outbreak resulted from re-emergence of the infection in this canine host species in Mpumalanga in 2008. In two outbreaks (in 2005 and 2010) dog rabies infections were associated with human deaths. These data demonstrate the public and veterinary health threat of rabies in South Africa and the region.

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*CONTACT DETAILS

OIE Rabies Reference Laboratory, Agricultural Research Council-Onderstepoort Veterinary Institute, P Bag X05, Onderstepoort, Pretoria, 0110, South Africa. Contact nos: +27 12 5299 439 (telephone), +27 12 5299 390 (fax) and e-mail: SabetaC@arc.agric.za.

THE VARIABLE SPECTRUM OF CLINICAL SIGNS ASSOCIATED WITH RABIES – A PICTORIAL PRESENTATION

Kotze, J.*

ABSTRACT

The clinical signs of rabies are often stigmatized and simplified to a few well known symptoms. Signs of aggression and hydrophobia are accepted as typical nearly pathognomic. In South Africa the most important research in this area was done at the Allerton laboratory using surveys of reported signs on submitted positive samples. This presentation will use an array of videos of actual clinical cases to support the evidence from the Allerton laboratory that the clinical signs can be extremely varied and often surprising.

THE RE-EMERGENCE OF BOVINE TUBERCULOSIS IN A CHACMA BABOON (*PAPIO URSINUS*) TROOP IN THE KRUGER NATIONAL PARK

De Klerk-Lorist*, L., Lane, E., Gij van Pittius, N. & Van Helden, P.

ABSTRACT

Bovine tuberculosis was initially diagnosed in the baboon troop of Skukuza Rest Camp in the southern district of the Kruger National Park in 1996. After an extensive test and slaughter exercise no new cases of BTB in baboons were reported during the next 14 years. A single sick baboon was destroyed by the local ranger in August 2010 and the necropsy revealed severe tuberculous disease. The origin of infection could not be determined at the time, but a surveillance program was implemented and a total of 25 baboons were captured from the area surrounding the living quarters and the VUSWA workshop. A semi-closed shed within the workshop area was indicated as being used as a sleeping facility and therefore possibly aided the spread of the infection. Complete necropsies were performed on all captured individuals, organs were fixed in 10% buffered formalin, blood samples were collected and stored and affected tissues were frozen for culture. The spleen and lungs were most commonly involved where granulomatous lesions were recorded in cases with macroscopic pathology. BTB infection was confirmed in 50% of the baboons by way of culturing the *Mycobacterium bovis* organism. It was also noted that non-tuberculous mycobacteria (NTMs) were cultured from all organs and lymphoid structures not affected by *M. bovis*. After the initial 4month capture period, several more baboons were destroyed on an *ad hoc* basis (especially damage causing animals (DCAs) within the rest camp and village areas), which were all necropsied and sampled. Two years after the re-emergence of BTB in the Skukuza baboon troop, the disease seems to have disappeared again with the help of environmental manipulation, population control as well as a test and slaughter policy.

KEYWORDS

Chacma baboon, *Papio ursinus*, bovine tuberculosis, *Mycobacterium bovis*, Skukuza, Kruger National Park, non-tuberculous mycobacteria

*CONTACT DETAILS:

Lin-Mari de Klerk-Lorist

P.O. Box 156

Skukuza

1350

(013) 735 5641

082 321 3301

EPIDEMIOLOGICAL EVALUATION OF AN ANTHRAX OUTBREAK AT PAFURI FROM NOVEMBER 2009 TO MARCH 2010 (KRUGER NATIONAL PARK)

Dekker, E.H*, De Klerk-Lorist, L., Van Dyk, D.S. & Bengis R.G.

ABSTRACT

During January 2010 one positive impala blood smear was received from Pafuri section. However, the entire Pafuri region is an anthrax endemic area. Then in February 2010 two more blood smears from impala and one from a nyala were positively identified, and this warranted closer inspection upon which numerous more carcasses were discovered, the majority being impala. All the cases occurred in the Makuleke Contractual Park, which is a part of the Pafuri ranger section north of the Levuvhu River. Unfortunately this outbreak was only discovered most likely more than a month after the majority of animals had died. Advanced decomposition hampered the efforts to try and determine the actual time of death. In order to determine the severity of such an outbreak it was necessary to collect samples from all available carcasses. Blood smears were made from fresher carcasses where possible, and older dry bone samples were collected for culture. Where the exact site of a carcass could be determined through indication of stains from bodily fluids which exited the orifices and then pooled on the ground, soil samples were collected as well.

Heavy rains caused abnormal erosion and flushing of all drainages and flooding of all the flood plains, opening and spreading long buried spores to the surface, where animals graze on the new shoots or drink from the newly formed ponds. Due to the fact that the animals were in poor condition and probably because of the lack of good grazing, they mostly likely engorged themselves on the new shoots emerging in the immediate vicinity of these ponds. This situation most likely enhanced the risk of them getting infected by inhaling the anthrax spores exposed by the running water, or the spores pushed up by the grass sprouts, or even those still stuck to the emerging grass. Because of these observations it is postulated that this area can act as a “contractor area” for anthrax. However, this statement can only be confirmed through longitudinal collection of soil samples in all drainages and pans in an attempt to isolate anthrax.

KEY WORDS

Anthrax, *Bacillus anthracis*, spores, concentrator area

*CONTACT DETAILS:

At Dekker
P.O. Box 12
Skukuza
1350
(013) 735 5641

SHUNI VIRUS, AN ORTHOBUNYAVIRUS CAUSING NEUROLOGICAL DISEASE IN HORSES AND WILDLIFE IN SOUTH AFRICA

Van Eeden, C.¹, Van Niekerk, S.¹, Steyl, J.², Williams J.², Swanepoel B. & Venter, M.^{1,3#}

SUMMARY

The cause of severe neurological disease in both humans and animals in South Africa remains largely undiagnosed. Horses are highly sensitive to some of these viruses and have been used as sentinels for the identification of arboviruses such as WNV associated with neurologic disease in South Africa. During the seasonal occurrence of common vector-borne diseases many horses have febrile, neurologic, and fatal infections for which the etiology remains undetermined. Our aim was to identify and characterise unknown viruses in cases of undiagnosed neurological disease in South Africa.

In 2009 we identified a brain isolate as Shuni virus (SHUV) in a horse with suspected viral meningoencephalomyelitis, using virus discovery techniques. SHUV-specific primers were then designed and used to test specimens from an additional 111 horses and 53 wildlife cases with neurological symptoms.

We identified Shuni virus by RT-PCR in 7 equine and 4 wildlife cases, including a rhinoceros, a buffalo, a warthog and a crocodile, presenting with neurologic disease. Phylogenetic analysis was used to further characterise the virus. Both equine and wildlife cases grouped together but showed geographical clustering distinct to the prototype strain from Nigeria. This virus has in the past been isolated from apparently healthy cattle, sheep and goats. Our study has assigned a disease association to this virus and suggests it may play a noteworthy role in encephalitis in South Africa thus emphasising the need for increased surveillance and disease characterisation in South Africa.

INTRODUCTION

Arboviruses are maintained in nature by haematophagous arthropods such as mosquitoes, ticks, *Culicoides* midges and sandflies, principally by biological transmission between susceptible vertebrate hosts. Many zoonotic arboviruses, are capable of causing major outbreaks, sometimes with severe morbidity and high mortality rates and are important emerging and re-emerging diseases(1). Several mosquito borne zoonotic viruses in the families' *Flavi*-, *Bunya*- and *Alphavirus* have emerged from Africa as new pathogens in previously unaffected regions and caused major epidemics and epizootics, including West Nile virus; Rift Valley fever and Chikungunya virus (2). Horses in particular are highly sensitive to WNV and certain alphaviruses in the USA and have thus also been targeted as sentinel animals in the identification of zoonotic arboviruses associated with neurological disease in South Africa (3). During the seasonal occurrence of more readily recognised vector-borne diseases such as African horse sickness (AHSV) and Equine encephalosis (EEV), many horses' exhibit febrile, neurological and fatal infections for which the aetiology remains unsolved.

The objective of this study was to identify potentially novel viruses associated with neurological disease in horses and wildlife in South Africa. In 2009 we identified Shuni virus (SHUV) in a horse with severe neurological symptoms (4), this prompted us to design SHUV specific assays with the objective to screen further cases of acute disease, so as to elucidate the role which SHUV may play in neurological disease in South Africa.

METHODS

Specimens from a further 112 horses and 53 other animals were sent to the Department of Medical Virology, University of Pretoria (UP) by the Onderstepoort Veterinary institute and the UP Faculty of Veterinary Science, Onderstepoort, as well as by veterinarians from around the country. The specimens were screened as appropriate for rabies virus in fatal cases.

SHUV-specific primers were designed based on the sequence of the prototype Shuni virus isolate (5). Sequence alignment of this strain with the Shuni isolate identified by our group in 2009 was carried out using the ClustalW subroutine, which forms part of the Bioedit program (6) to identify suitable areas for binding.

¹Zoonosis Unit, Faculty of Health Sciences, University of Pretoria, South Africa.

²Department of Paraclinical Science, Faculty of Veterinary Science, University of Pretoria, South Africa.

³Centre for Respiratory diseases and meningitis, National Institute for Communicable diseases, Sandringham, South Africa

Presenting author: Charmaine van Eeden

Contact author: Marietjie Venter

Zoonosis Research Unit,

Room 2-72 Pathology Building

5 Bophelo road

Pretoria

0001

Tel: 012 319 2287; Fax: 012 325 5550; Email:marietjiev@nicd.ac.za

RT-PCR products were sequenced and edited using Sequencher v4.6. Neighbor joining trees were generated with Mega version 4 using 1000 bootstrap analysis and the p-distance algorithm following sequence alignment. Maximum likelihood trees were generated using PHYML using 100 bootstrap replicates. P-distance analyses were carried out for nucleotide and amino acid sequences using Mega v4 (7).

RESULTS

A total of 112 horses and 53 other animals with unexplained fever and/or neurological disease were screened with the nested SHUV-specific PCR. SHUV was identified in 7 horses, 2/26 (8%) with unexplained fever, and 5/86 (6%) with nervous disease and in 4 other animals with nervous signs 4/53 (7.5%).

SHUV infections were identified during January, April, May, July and August in the Gauteng, Limpopo and Northern Cape Provinces. Three of the 5 horses which manifested nervous disease had to be euthanized in extremis. Clinical signs included anorexia 5/7, ataxia 4/7 muscle tremors 3/7, depression 3/7, recumbency 2/7, and paralysis 2/7. The 2 horses with febrile disease and 2 with mild nervous disease made full recoveries. Of the four other animals, two succumbed to disease and two were euthanized for humane reasons. Clinical signs included muscle tremors 1/4, recumbency 1/4 and paralysis 4/4.

DISCUSSION

SHUV was first isolated in the 1960s from cattle and sheep in abattoirs; *Culicoides* midges tested in arbovirus surveys and in one instance from a febrile child in hospital, in Nigeria (8). Subsequently, the virus was isolated from pools of *Culex theileri* mosquitoes caught near Johannesburg, and from cattle and a goat in KwaZulu-Natal Province, South Africa (9, 10). In 1977, the virus was isolated from the brains of two horses that succumbed to nervous disease, one in South Africa and one in Zimbabwe (11, 12). Specific Shuni virus diagnostic tests were however never developed and no further investigations were undertaken to determine the importance of this virus as a cause of neurological disease in humans or animals. Identification of this virus from a horse with severe neurological symptoms as part of a virus discovery project in 2009 prompted us to design specific Shuni virus primers and screen further cases of acute disease.

Two SHUV specific assays were developed to determine the role of this virus in neurological disease. Both the nested RT-PCR and nested real-time assays were specific, reproducible and sensitive to the detection of low level virus in clinical specimens. Over a period of 18 months we were able to identify a further 10 SHUV cases, eight of which were associated with significant neurological symptoms including tremors, convulsions and paralysis. These findings suggest that the role of SHUV as a pathogen may be underestimated, and that it should be investigated routinely as a possible cause of unexplained nervous disease of humans and other animals, not only in South Africa but across the African continent.

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DATA ANALYSIS OF ENQUIRIES TO A SOUTH AFRICAN RADIO PROGRAMME ON ANIMAL MATTERS

AS Cloete^a and JD Grewar^b

INTRODUCTION

Over a period of six years, 2 385 letters and e-mail messages were submitted to a weekly veterinary information programme, DiereManiere, meaning “animal manners”, on the South African Broadcasting Corporation (SABC) national Afrikaans cultural service, Radio Sonder Grense (RSG), meaning “radio without borders”¹⁰. A total of 1 839 (77%) of this correspondence contained one or more questions on veterinary and animal matters. Of these enquiries a total of 892 (48.5%) were answered during a period of five and a half years by an appointed veterinary presenter. Specialists and experts were also incorporated to add value to the information supplied.

The measured daily DiereManiere listener figures ranged between 293 000 and 332 000 over 5 consecutive years. The audience mostly comprised Afrikaans speaking adults over the age of 35 years, with significant listener concentration in the Western Cape and Gauteng provinces. The audience also represented the more affluent portion of the South African population.

The purpose of the study is to estimate the prevalence of the most common complaints regarding animals in South Africa, and compare it to the prevalence of disorders of dogs and cats examined at private veterinary practice, as published internationally.

MATERIAL AND METHODS

Listener figures were collected by means of an international standard measurement, namely Radio Audience Measurement Survey (RAMS),⁹ during the 3rd quarters of 2004-2008.

Recruitment of correspondence to the radio programme was voluntary and the detail of information supplied according to the writers prerogative, accounting for the convenience sampling method implemented. Where no problem or specific enquiry was stated the animal was assumed to be healthy and not included in the data set. This accounted for 33% of all correspondence received (n=564). Lund et al (1993) reported that about 7% of dogs and 10% of cats examined by practitioners during their study were considered healthy³. The data from the remaining 1 839 letters and e-mails containing veterinary enquiries were collated in a Microsoft Access database, taking all the relevant available information into account. A description of the problem(s) was captured, ranging from a vague term such as hair loss, to a specific reported diagnosis such as ringworm. Described problems (n=1 943) were categorised, for example behaviour, skin and medicine amongst others. In the case of larger categories, the problems were sub-categorised into the most specific problem category, for example alopecia for skin problems or respiratory and nervous system subcategories for the medicine category. If an enquiry related to information required, rather than a problem with the animal, it was categorised separately, for example information on the gestation period of a bitch was categorised under General Reproduction, whereas a uterine infection sorted under Medicine Reproduction and the spaying of a bitch under Surgery Sterilisation.

Apart from the identified problem(s) the correspondence often supplied information such as species (n=1837), breed (known in 73% of data set) including 62 dog breeds, gender (65% of data set) and age of the animal (34% of data set), the date of writing (87% of data set) and location (46% of data set) from which approximate geographic location was acquired. The enquiries concerned mostly companion animals within the canine (68%), avian (14 %) and feline (13%) species, apart from several other livestock and exotic pet species.

RESULTS AND DISCUSSION

The results were structured according to a loose interpretation of the agent-host-environment epidemiological triad, in order to clarify possible tendencies and associations.

THE ENVIRONMENT:

Measured over 5 years the average number of DiereManiere listeners comprised around 300 000 per week from a total South African radio audience of approximately 30 million per week. This was a stable 1% of the total South African radio audience over the period, with the highest 1.1% in 2005 (332 000/30.656 million) and lowest 0.9% in 2008 (295 000/31.303million). It was estimated that the total Afrikaans speaking radio audience is around 3.588 million people, which translates the DiereManiere audience to almost 8.4% of the Afrikaans speaking radio audience in South Africa^{8, 9, 10 and 11}.

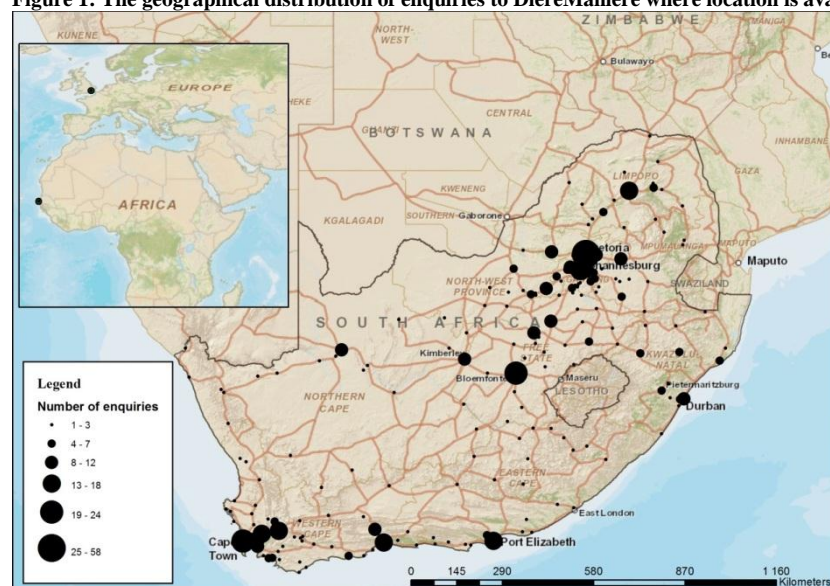
a&b Western Cape Department of Agriculture, Veterinary Services, Private Bag X1, Elsenburg, 7607.

a Tel no (021) 808 5254, Fax (021) 808 5126, E-mail: AnnelieC@elsenburg.com

b Tel no (021) 808 5056, Fax (021) 808 5126, E-mail: JohnG@elsenburg.com

The age distribution of the majority of the measured DireManiere audience ranged between the age categories of 35-49 years and 50 years and older. The Living Standard Measurement (LSM) of the listener population ranged from 7-10, which is the higher income part of the population. RAMS, however, only accessed traditional radio listener figures, excluding all internet radio streaming audience components. Electronic correspondence grew considerably in number since the implementation of an in-studio e-mail system via the internet towards the end of 2006. The geographical distribution of the correspondence received (see Figure 1) was very similar to the geographical distribution of the Afrikaans speaking families as published by Statistics South Africa in 2011.

Figure 1: The geographical distribution of enquiries to DireManiere where location is available (65% of the data set).



The measured age distribution of the DireManiere audience (from RAMS statistics) corresponds to the age profile of writers of enquiries to the radio programme and this is also comparable to the average reported age of 43.4 years in private practice¹. Where the sex of the writers could be ascertained (n=1584) 80% were females, which is higher than the 68% female respondents reporter in private veterinary practice by Lue et al (2007)¹. This difference could possibly be attributed to a larger component of female radio listeners. The tendency of a female or mother in a family taking responsibility for the care and wellbeing of the pets could explain the higher representation by females in general.

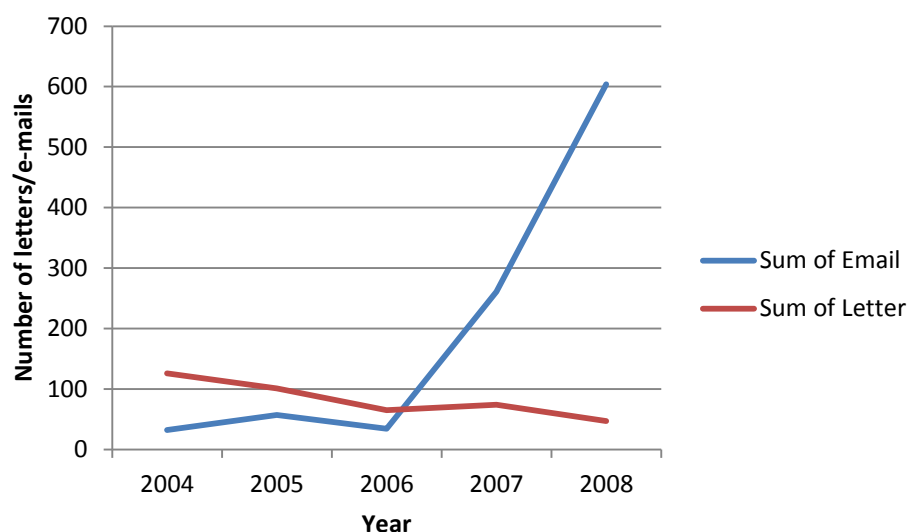
Pet owner demographics in private veterinary practice are not commonly reported, but according to the same study (Lue et al, 2007) pet owners were slightly more affluent than the overall US population¹. The higher income listener population (RAMS) therefore compares favourably to the average client profile in private veterinary practice. Nearly 58.3% of US households owned some type of pet in 2001.^{2, 12} According to Lue et al (2007) "pet owners can be stratified into homogeneous "price sensitivity" groups namely: price unconscious (38%), price conscious (43%), and price sensitive (19%).¹ Whereas price-unconscious owners are willing to obtain veterinary care at any cost without thinking about the price, those who are price conscious take price into account in their decisions."¹ One would expect that the offering of free information and advice would encourage price conscious and price sensitive listeners who are not always able to afford veterinary care¹. Most veterinary clients pay the entire bill for services rendered in contrast to health insurance clients². John Volk goes as far to state that "pet owners fear the bill they'll get at the veterinary clinic"⁶. The perceived cost of veterinary care would thus stimulate interest in free advice.

Volk also reports that "pet owners do not see the value of preventive check-ups", which touches on another plausible explanation for the interest in radio veterinary advice, being a need for better pet owner education. Veterinary clients, "are not well informed consumers of veterinary healthcare services"^{2, 13}. The veterinary consumer needs information to make informed health care decisions.² Successful pet health care and treatment is often dependent on client adherence to veterinary advice and compliance by following recommendations for preventive care as well as treatment protocols for specific diagnoses. Such compliance requires an informed consumer^{2, 14}. In the Lue et al study (2007) 13% of respondents reported that their current pet was their first, meaning approximately 1 in 8 owners had little or no experience caring for a dog or cat.¹ This suggestion is supported by the DireManiere data, where the General category, comprising mostly of general pet care and basic knowledge (including diet, dental care, hygiene, reproduction, as well as species and breed information) forms almost 20% (n=352) of the enquiries. In Figure 3 the proportion of the 6 different problem categories from the DireManiere data is illustrated. Awareness and education can increase perceived value and subsequently the demand and willingness to pay for services.¹ Consumers seek health care information from a variety of sources, including the radio. This may encourage a veterinary visit, when the

individual determines whether vague symptoms are serious and require further exploration.^{2, 15} Information could also help an owner cope better with anxiety, frustration, or confusion following a pet's diagnosis and ultimately help the owner make decisions about the pet².

The mode of communication is also explored as ease of access to the presenter was vastly improved by internet radio (audio streaming). The proportion of letters (n=565) is 31% of the total correspondence received with e-mail messages (n=1272) the remaining 69%. The facility of allowing listeners to send e-mails directly to the studio during broadcastings significantly increased the enquiry rate since its introduction during 2007, as illustrated in figure 2. This is most probably due to the ease of electronic correspondence against traditional mail correspondence and probably includes partly the non-traditional radio listener audience using radio internet streaming, which is not included in the RAMS measurements. The e-mails received from outside the country (see figure 1) confirm the radio internet streaming audience. The Bayer Veterinary Care Usage Study, 2011 revealed that nearly all pet owners use the Internet – E-mail, Facebook and Google amongst others.⁷

Figure 2: The sum of letter versus e-mail correspondence per year for 2004 to 2008 (86.8% of the data set had a date of writing) Note the dramatic increase in e-mail correspondence from 2006 onwards with a corresponding decrease in letter correspondence from that point onwards.:



THE HOST:

In this study a considerable difference between the number of dog (n=1245) enquiries (68%) and the number of cat (n=231) enquiries (14%) was observed. A similar tendency was reported by Lue et al (2007) where it was found that cats were substantially underserved, compared to dogs. Lue et al deduced that it was likely the result of the lack of owner attachment and understanding of the care cats should receive. Their findings revealed that 79% of cat owners paid nothing for their cats, compared with 43% of dog owners.¹ Of those who did pay for their pets, the cat owner spent on average 60% less on their cat's veterinary care, compared to veterinary care expenditure by dog owners.¹ Households with dogs also had higher median incomes than households with cats¹. The species discrepancy was also confirmed by the Bayer Veterinary Care Usage Study (2011)⁶, which reported 13% more cats than dogs in the US, but cats are the minority of patients in most practices⁷.

For enquiries regarding dogs less than 21% did not specify the breed. In contrast 90% of cat related enquiries did not specify the breed. For 79% of the dog enquiries, the breed (n=987) was indicated, with a total of 59 distinct dog breeds and 3 separate cross-breed categories, namely small and large crossbreeds according to a direct breed linkage mentioned and a general cross breed category where no indication was given. The Jack Russell Terrier was the highest represented breed (9%), followed by the Dachshund (7%) and the South African Boerboel (6%).

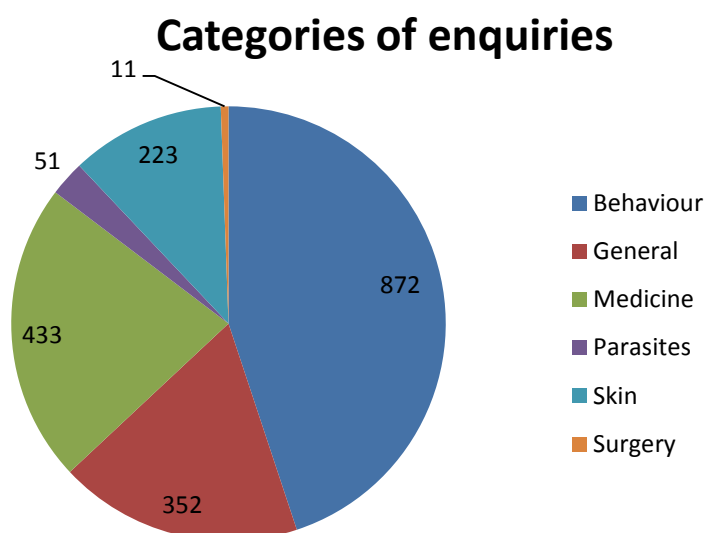
In 35% of the DierManiere data set the age of the animal is available. The average ages of the dogs and cats are respectively 4.01 and 5.05 years. This is slightly lower than but correlates with data from the Lue et al study (2007) which reported that cats (average age 5.9 years) were approximately half a year older than dogs (average age 5.5 years)¹.

THE AGENT:

Analysis of the enquiries or problems (n=1942) proved very interesting, with the most common problems involving behavioural issues (45%) (n=872). See figure 3 for the different categories and their proportions. Medical enquiries comprised 34% (n=656) of all questions, with skin problems (n=223) a third of these and therefore categorised

separately. General non-medical (n=352) enquiries form another 18% of cases and this can be equated to the bulk of the education and information needs of the population. An unexpected almost 3% of enquiries relating to parasites (n=51) can possibly be attributed to the intense worry during particularly the warmer seasons. Surgical enquiries (n=11) were the lowest represented independent category at 0.6%. This could possibly be related to adequate owner education in the private practice setting for surgical procedures.

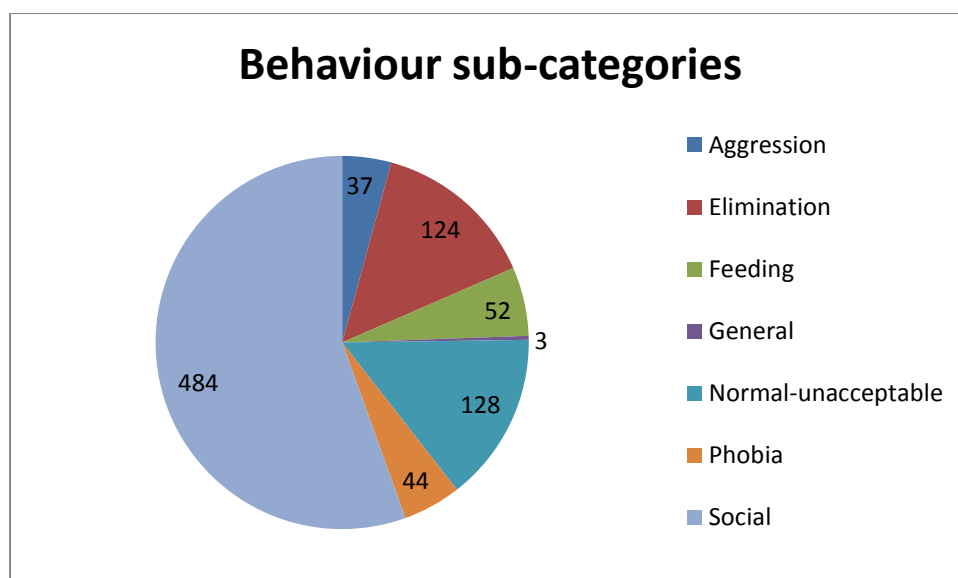
Figure 3: The proportion of problem categories from the DierManiere data. By far the majority of enquiries fell within the behaviour category (45%) highlighting the issues listeners had regarding this category.



Birds and exotic pets had a larger general enquiry component compared to the other species, which could be explained by the higher number of general species and breed enquiries of new or inexperienced owners.

The most common behavioural enquiries (n=869) concerned social interaction (55%), which include both negative and positive interaction within species and also intra species with male animals more likely to be involved than bitches and queens. This is followed by normal, but unacceptable behaviour (15%), for example barking, which is very normal for a dog but might be totally unacceptable to people. These questions are probably not perceived by owners to justify a veterinary consultation fee and the radio programme is therefore seen as a preferred medium to enquire from. Elimination behaviour (14%) included inappropriate excretion, which Borchelt (1986) reported as “Problem urination is the most frequent feline behavioural symptom for which veterinary consultation is sought”¹⁶ and in 1975 Campbell claimed “Problems in housebreaking (potty training) are the most frequently reported behavioural problem in dogs”¹⁷. Elimination was the second category where sex seemed to be a predisposing factor, as dogs and tomcats were almost twice as often implicated than bitches and queens. Feeding behaviour (6%), phobias (5%) and aggression (4%) comprised the rest of the behavioural issues. Interestingly, 17% of enquiries related to the Boerboel dog breed were about aggression. See figure 4 for a graphical illustration of the behavioural problems reported in the data set.

Figure 4: Sub-categorisation of the largest problem category in the Diermaniere database, namely behaviour. Note the majority of behaviour enquiries regarded social behaviour.

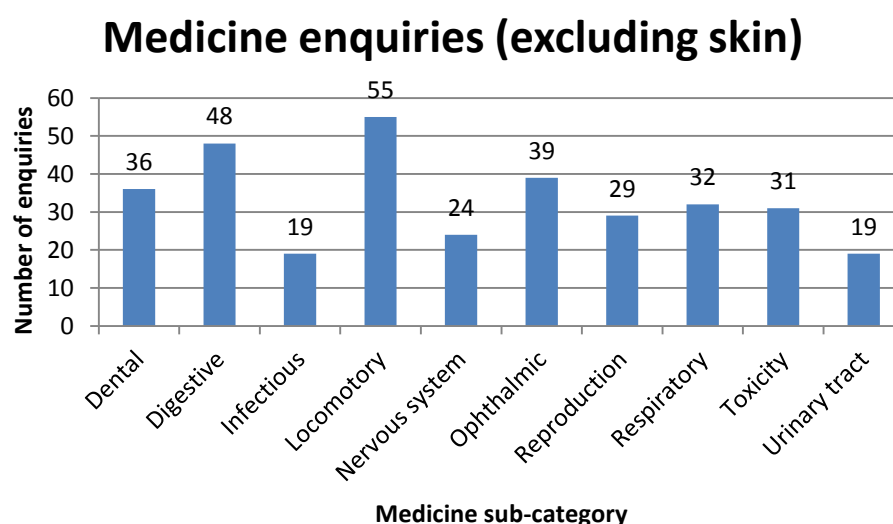


A seasonal distribution could be illustrated in this category only with an increase in behavioural enquiries after every school holiday period, which could possibly be attributed to an increase in human-animal interaction time.

A third of the Medicine category comprised of skin enquiries (n=206). The most common reported skin complaint was pruritus (34%), followed closely by alopecia / feather loss (29%). Feather loss in birds, which is caused by a variety of possible aetiologies amongst which behaviour, was the 3rd most commonly questioned issue in pet birds, after questions regarding diet and social interaction. Pruritus and dermatitis questions seemed to be more frequent during the summer months, which might be an indication of external parasite involvement, and specifically fleas, which are deemed as the primary causes for pruritus in dogs⁵.

The most common enquiries in the rest of the Medicine category differs slightly from the reported findings by Lund et al (1993) that dental calculus and gingivitis were the most commonly reported disorders. Lund stated that: “many conditions were common to both species (for example flea infestation, conjunctivitis, diarrhoea, vomiting). Dogs were likely to be examined because of lameness, disk disease, lipoma, and allergic dermatitis. Cats were likely to be examined because of renal disease, cystitis, feline urologic syndrome, and inappetence”³. Most of the problems mentioned by Lund are, however, also present in the DiereManiere data set with locomotory (n=55) and digestive (n=48), problems as the most common sub-categories. Figure 5 demonstrates the top 10 medicine categories in the DiereManiere data set, excluding skin related enquiries.

Figure 5: The most common enquiries regarding Medicine, apart from skin, in the DiereManiere data set.



The General category (n=352) included amongst others breed (37) and species information (70), deterrents for problem animals, for example how to keep the neighbour's cat out of a bird-friendly garden (14), hygiene and general wound care (12), primary animal health care (11), tail docking (3), which was proclaimed an unethical procedure during this period and attracted some interest, traveling with pets (13), welfare issues (11) and information regarding the veterinary occupation (5).

CONCLUSIONS

Apart from a slightly higher percentage of females, the listener population requiring veterinary advice was found to be comparable to the client base in general veterinary practice. A bias towards the interest of the veterinary presenter, the name of the radio programme, as well as a considerable need for inexperienced pet owner education and the fear of a veterinary bill for a non-medical problem could explain the exceptionally large component of behavioural and general enquiries in this study (45% and 18% respectively). The patient base was very similar and the extent and distribution of the most common medical needs were nevertheless found to be similar to that in private veterinary practice.

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I want to thank the personnel of RSG with whom I had the honour of working for more than 5 years, as well as the management of the Western Cape Department of Agriculture who afforded me the opportunity to present *DiereManiere*. The support and assistance from numerous specialists and private veterinarians, as well as the South African Veterinary Foundation, who sponsored the participation of specialists hosted on the programme. Ms Ilze Bothe is acknowledged for her assistance with data capturing from the correspondence, Dr Marna Sinclair for technical support and Dr Lesley van Helden for editorial assistance.

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VALUE OF ADDING NURSES TO VETERINARY TEAMS FOR HEALTH COMMUNICATION ABOUT ZONOTIC BOVINE BRUCELLOSIS

Woods, P.

ABSTRACT

Bovine brucellosis has been a re-emerging zoonoses amongst small-scale farmers in Zimbabwe since 2001 when Government veterinary services discontinued free vaccinations due to financial constraints. Following land invasions where uncontrolled movement of cattle led to brucella-positive cattle entering naive herds, abortions were reported although the cause was unknown. This project hypothesised that increasing communities' knowledge about severity of and susceptibility to brucellosis would lead to calf vaccination and adoption of safe milk practices. Initially we formed veterinary teams who conducted health education sessions for farmer groups through a dairy cooperative network. This led to large, significant increases in awareness and improved payment for calf vaccination. However, although an increase in knowledge about the dangers of consuming raw milk was recorded, there were no corresponding behaviour changes. Only 5% of all the households sampled boiled milk before consumption with a significantly lower percentage of "knowledgeable" farmers who consumed raw milk compared to the "Unaware of Brucellosis" group (27% and 48% respectively). Despite the intervention, some farmers were still not making informed choices to decrease the risk of their livestock or families being exposed and infected with brucellosis. We added nurses and environmental health technicians to the education teams. This led to improved information dissemination, an increase in knowledge and boiling milk behaviour. Follow-up household interviews (n=170) recorded calf vaccination rates further increased and more free serological tests requested. The first human cases were detected in the district. More women attended the nurses' talks than previously where mostly men attended the veterinary talks. The cross-disciplinary teams were more effective in health communication and subsequent behaviour changes. This project provides evidence and recommendations for future community-directed zoonosis control when farmers are required to participate and pay for any procedures.

MOLECULAR PREVALENCE OF *ANAPLASMA MARGINALE* (RICKETTSIALES: ANAPLASMATACEAE) IN NGUNI AND LOCAL CROSSBRED CATTLE UNDER THE LOW INPUT PRODUCTION SYSTEM IN SOUTH AFRICA

Munyaradzi C. Marufu¹, Michael Chimonyo^{1*}, M.S. Mtshali², Kennedy G. Dzama³

ABSTRACT

Control of tick-borne diseases depends on the formulation of apposite control stratagems based on accurate epidemiological data on disease prevalence in particular production systems. The objective of the current study was to establish the molecular prevalence of *Anaplasma marginale* in cattle in the low input production systems in South Africa. Body condition score (BCS), packed cell volume (PCV), tick infestation levels and the molecular prevalence of *A. marginale* was determined by PCR from the blood of cattle from different production systems, genotypes, age groups and both sexes in the Eastern Cape Province of South Africa. Molecular prevalence of *A. marginale* was higher ($P < 0.05$) in cattle on the small scale farms (85.7 %) than those in the communal areas (30.6 %). High levels of infection in calves and immunity in adult cattle, coupled with the absence of clinical disease were observed and reflective of a situation of endemic stability to bovine anaplasmosis. Cattle in the small scale farms, young animals and those with low BCS had higher ($P < 0.05$) odds of being infected by *A. marginale*. Nguni cattle suffered less severe losses from and were more resilient to *A. marginale* infection than local crossbreds. Further elucidation of the genotype associated mechanisms of resistance to anaplasmosis in indigenous Nguni cattle is required.

Keywords: Communal, Endemic stability, Polymerase chain reaction, Small scale

INTRODUCTION

Ticks and tick borne diseases (TBD) cause widespread morbidity and cattle mortality in the low input cattle production systems (Hesterberg et al., 2007; Marufu et al 2010; 2011). In particular, bovine anaplasmosis, caused by the rickettsial haemoparasite *Anaplasma marginale* and transmitted to cattle biologically by *Rhipicephalus (Boophilus)* ticks and mechanically by flies and fomites (Aubrey and Geale, 2010) is one of the most common causes of cattle mortalities in low input farming areas in South Africa (Mapiye et al., 2009; Ndou et al., 2010). The disease results in considerable economic losses to the cattle industry through decreased production, lowered working efficiency of cattle and increased veterinary costs and deaths (Kocan et al., 2003). Cost-effective control of bovine anaplasmosis depends on the availability of accurate prevalence data which is scanty for cattle in low input production systems. Disease prevalence studies are tools that can be used to provide such data and to demonstrate the risk or impact of anaplasmosis in local and global economies (Awad et al., 2011).

Epidemiological field studies using serological tests that detect antibodies reactive with tick borne haemoparasites in cattle in communal areas have shown that the Nguni genotype has a lower sero-prevalence of *A. marginale* than the local crossbreds (Nguni x exotic crosses) (Marufu et al., 2010). It was thus postulated that the Nguni genotype has a superior resistance to bovine anaplasmosis than local crossbreds. Serology based techniques have a major disadvantage of cross reactivity between species (Kocan et al., 2010). Polymerase chain reaction (PCR) based detection methods have been developed that are extremely sensitive and specific in the detection of *A. marginale* infections in cattle (de la Fuente et al., 2005; Molad et al., 2006). Accurate data on prevalence of bovine anaplasmosis obtained by such highly sensitive and specific molecular techniques are crucial not only for developing appropriate control measures and but for providing an understanding of host resistance in different cattle genotypes. Little work has been done on the molecular prevalence of bovine anaplasmosis in the different cattle genotypes reared under low input production systems in South Africa thus warranting further investigations.

A strong relationship exists between nutrition and disease infections in ruminants. Animals with higher levels of protein and/or energy are better able to control the establishment of new diseases and reduce fecundity of existing pathogens (Coop and Holmes, 1996). Superior nutritional status could likely contribute to increased resistance to TBD infection in indigenous cattle breeds and yet its benefits have not been assessed in indigenous cattle in the low input system. It is

¹Department of Animal and Poultry Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3201, South Africa.

²Research and Scientific Services Department, National Zoological Gardens of South Africa, P.O. Box 754, Pretoria, 0001, South Africa.

³Department of Animal Sciences, University of Stellenbosch, Private Bag X1, Matieland, South Africa.

*Corresponding author Telephone: +27 33 260 5477 Fax: +27 33 260 5067 Email: chimonyo@ukzn.ac.za.

essential therefore to investigate the influence of *A. marginale* infection on nutritional performance measures such as body weight, body condition score and packed cell volume in cattle under low input production. The current study was conducted as an initial step in a broader investigation of mechanisms of resistance to *A. marginale* in the indigenous Nguni cattle genotype. The objective of the current study was to determine the molecular prevalence of *A. marginale* in Nguni and local crossbred cattle in the low input farming system. It was hypothesised that the prevalence of bovine anaplasmosis is lower in Nguni than in local crossbred cattle reared in low input farming areas of South Africa.

MATERIALS AND METHODS

STUDY SITE

Blood sample collection was conducted in June 2010 at communal areas and small scale farms located in the Sakhisizwe Local Municipality of Chris Hani District Municipality in the Eastern Cape Province, South Africa (Figure 1). Stratified random sampling based on the production system was used to select the three communities and three farms sampled in the study. Small scale farms that were owned by beneficiaries of the government's land restitution programme and the surrounding communal areas were chosen. Land restitution is a government-initiated programme of redistributing commercial agricultural land to benefit previously disadvantaged farmers (Mapekula et al., 2009).

The study area is located on 27° 50' East and 31° 27' South, and composed of a sour rangeland in which forages have low nutritive value and are largely unpalatable during the dry season (Ellery et al., 1995). The most common grass species are *Themeda triandra*, *Sporobolus africanus* and *Microchloa ciliate*. *Euryops pyroides*, *Chrysocoma ciliate* and *Dyspyrose scrabrida* are the common bush species in the areas (Lesoli, 2008). The study area lies at an altitude of 850 – 1900 m and receives moderate average annual rainfall of 600–800 mm which mostly occurs during the wet season (November to April). Average temperature is highest in the hot wet season (20 °C) and lowest in the cool dry season (11 °C). Cattle graze on rangelands throughout the year.

STUDY ANIMALS

All experimental procedures were specifically approved for this study by the University of KwaZulu-Natal Animal Ethics Research Committee (Reference number 097/11/Animal) and were in compliance with internationally accepted standards for animal welfare and ethics. A total of 149 clinically healthy cattle classified according to genotype (70 Nguni and 79 local crossbred), sex (68 male and 81 female), age (72 less than 2 years old and 77 older than 2 years old) and production system (75 communal and 74 small scale) were selected and sampled in the study. The animals were selected on the basis of the owners' willingness to participate in the study. Small scale farmers dipped their cattle four times in the wet season, and twice in the dry season (May to October). Communal farmers depended on State Veterinary Services for dipping which was conducted fortnightly in the wet season and monthly in the dry season at the communal dipping tank. All animals grazed on natural rangelands throughout the study period.

DETERMINATION OF BODY WEIGHTS, BODY CONDITION SCORES AND TICK INFESTATION LEVELS

For each animal, the body weight was estimated using a cattle weigh-band whilst visual assessment of the body condition was made using the five-point scoring system (1 – very thin and 5 – obese). Engorged adult ixodid ticks were counted from the whole body of each animal. The tick counts were classified into three infestation levels as follows low (<30 engorged adult ticks on the whole body), moderate (> 30 – 50 engorged adult ticks on the whole body) and high (> 50 engorged adult ticks on the whole body).

BLOOD COLLECTION

Blood samples for PCV determination and *A. marginale* detection were collected from the 149 study animals. The cattle were held in a crush while the blood samples were collected from the tail vein using an 18 gauge needle into two well labelled, blood tubes containing EDTA for each animal. One blood tube was stored at 4 °C and used for PCV determination while the other was stored at -20 °C and used for DNA extraction for each animal.

DETERMINATION OF PACKED CELL VOLUME (PCV)

For the determination of PCV blood which was stored at 4 °C was transferred into micro-haematocrit tubes and centrifuged in a micro-haematocrit centrifuge (Gemmy Industrial Corp.) for 3 minutes. Reading of the PCV was performed on a Micro-haematocrit Reader Scale.

DNA EXTRACTION AND AMPLIFICATION

For each blood sample, DNA was extracted from 100 µl of EDTA blood using the ZR Genomic DNATM- Tissue MiniPrep Kit (Zymo Research, California, USA) at the National Zoological Gardens Parasitology Laboratory. The DNA was re-suspended in sterile distilled water and stored at -20 °C until used in PCRs. The 1733 F: 5'-TGTGCTTATGGCAGACATTTC-3' and 2957 R: 5'- AAACCTTG TAGCCCCAACTTATCC-3' genes were amplified from 1 µg *A. marginale* DNA by PCR using 10 pmol of each primer (1733 F and 2957 R) in a 25 µl volume (12.5 µl DreamTaqTM Green PCR Master Mix, 10 pmol of each primer and 1 µg of Template DNA) in the BOECO Thermal cycler (Hamburg, Germany). The amplification cycles, following an initial deanturation at 94 °C for 3 minutes, consisted of 35 cycles of 1 minute at 94 °C, 1 minute at 60 °C and 1 minute at 72 °C, followed by a final cycle with a 10 minutes extension step at 72 °C. Amplified PCR products were separated in 1% TBE (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) agarose gel, using GeneRulerTM 1Kb Plus DNA ladder (Fermentas Life Sciences, USA). Gel was visualized and photographed under UV illumination after Biotium GelRed acid staining.

Molecular prevalence of the three haemoparasites was calculated as: $P = \frac{d}{n} \times 100$

where P = prevalence; d = number of animals that test positive for DNA to the haemoparasite species; and n = total number of animals tested for the haemoparasite (Thrusfield, 1995).

STATISTICAL ANALYSIS

Data were analysed using SAS (2006). To test for normality, the data was subjected to univariate analysis. Data for BCS were not normally distributed and were subjected to square root transformation to confer normality. The effect of production system, genotype, age, sex, infection with *A. marginale* and their interactions on the body weight, BCS and PCV were determined using PROC GLM (SAS, 2006). Chi square test was used to determine the associations between tick infestation level or molecular prevalence of *A. marginale* and production system, genotype, age and sex. Logistic regression was used to determine the odds of infection with *A. marginale* between production systems, breeds and sexes and across seasons and age groups.

RESULTS

MOLECULAR PREVALENCE OF A. MARGINALE

A sample photograph of the agarose gel plate after exposure to ultraviolet light, with some positive reactions, is shown in Figure 2. Of the 149 cattle sampled for molecular prevalence in the study, 88 (59.1 %) were infected with *A. marginale*, 42 (47.7 %) being of the Nguni genotype, while 46 (52.3 %) were local crossbreeds. There was an association ($P < 0.05$) between molecular prevalence of *A. marginale* and production system ($\chi^2 = 46.8$). Cattle in the communal production system had a lower ($P < 0.05$) molecular prevalence of *A. marginale* than those in the small scale system (Table 1). There were no associations ($P > 0.05$) between molecular prevalence of *A. marginale* and genotype.

PROBABILITY OF INFECTION WITH A. MARGINALE

Logistic regression showed that the odds of an animal being infected by *A. marginale* were higher ($P < 0.05$) for cattle in the small scale farms than in the communal areas (Table 2). Young animals (below 2 years old) had higher odds of infection by bovine anaplasmosis than older animals. Cattle with high BCS had lower odds of infection with *A. marginale* than those with low BCS.

BODY WEIGHTS, BODY CONDITION SCORES AND PACKED CELL VOLUME

The interaction between infection with *A. marginale* and genotype and between infection with *A. marginale*, genotype and production system had a significant ($P < 0.05$) effect on the body weight of the study animals. Uninfected local crossbred cattle had higher ($P < 0.05$) body weights than their infected counterparts in the communal and small scale areas, while both infected and uninfected Nguni cattle had similar ($P > 0.05$) body weights in both production systems (Table 3). The interaction between infection with *A. marginale* and genotype had a significant effect on the BCS of the study animals. Infected and non-infected Nguni cattle had similar mean BCS which were higher ($P < 0.05$) than those of non-infected local crossbred cattle while infected local crossbred cattle had the least mean BCS (Table 4). The interaction between infection with *A. marginale* and age had a significant effect on the packed cell volume of the study animals. Young infected animals had significantly lower ($P < 0.05$) PCV than young non infected, older infected and older non infected animals (Table 5).

TICK INFESTATION LEVELS

Tick infestation levels were associated with management type ($\chi^2 = 14.2$; $P < 0.05$). Cattle on the small scale farms had higher ($P < 0.05$) tick infestation levels than those in the communal areas. Genotype, age and sex were not significantly associated with tick infestation levels in cattle.

DISCUSSION

The overall molecular prevalence of 59.1% observed for *A. marginale* in the current study was moderate and similar to that reported by Mtshali et al. (2007) for cattle in the Free State Province. However, it was higher than that reported previously in a sero-prevalence study for communal cattle in the Eastern Cape by Marufu et al. (2010). Differences in the prevalence between the two studies could be attributed to the inclusion of small scale farms in the present study which are known to have different tick and TBD control strategies to communal farmers (Bryson et al., 2002; Rikhotso et al., 2005). The differences in the specificity of the serological methods used by Marufu et al. (2010) and the molecular techniques used in the current study could also explain the disparities in the observed prevalence. Molecular diagnostic techniques used in the present study detect active infection and thus provide more accurate temporal data on disease prevalence status in the study area than serological tests used in the previous study which only detect exposure to infection which is not necessarily current.

Observed differences in molecular prevalence of *A. marginale* in the small scale and communal production systems were likely influenced by variations in the control and infestation levels of ticks, the major biological vectors of *A. marginale*. Communal farmers rely heavily on the government subsidised dipping programme (Rikhotso et al., 2005) while small scale farmers utilise their own resources to purchase acaricides (Mapiye et al., 2009). In the present study acaricides were applied once fortnightly in the communal cattle herds and approximately once monthly and according to tick infestation levels in the small scale areas. Increased host (cattle) - vector (tick) contact times result in increased TBD transmission rates (Muhanguzi et al., 2010) which could have caused the observed higher odds of infection with *A. marginale* in the small scale production system.

Despite higher prevalence of *A. marginale* in the study area, clinical disease in cattle was not observed in the study animals, which might reflect a situation of endemic stability. Endemic stability is an epidemiological state, in which clinical disease is scarce despite high levels of infection in the population (Coleman et al., 2001; Jonsson et al., 2012). Such a situation arises if the force of infection is high enough that acquisition of functional immunity occurs in the majority of the population at a relatively young age, when the disease is often mild compared with disease in older animals (Hay, 2001). In the present study, younger animals had a higher prevalence of *A. marginale* and higher odds of infection than adult cattle, which could likely have contributed to the development of endemic stability. To minimize the direct effects of ticks in cattle while conserving endemic stability, farmers have to maintain tick loads above a minimum threshold (Eisler et al., 2003). The minimum threshold for ticks on cattle populations in low input areas, however, is still vague and needs to be established.

The observed absence of clinical disease in young animals in the current study despite high odds of infection reinforces the view that young animals are less susceptible to clinical bovine anaplasmosis (Kocan et al., 2003). The cause of reduced susceptibility to anaplasmosis in young animals is not well understood. Variation in susceptibility to *A. marginale* between young and old cattle is thought to arise from the differences in the dominant immune response (innate or

acquired) to *A. marginale* infection in the two categories (Muhanguzi et al., 2010). Young animals however most likely become persistently infected or “carriers” for life (de La Fuente et al., 2010) despite their higher immunity to infection. Low input farmers need to be wary of persistently infected animals as they serve as reservoirs of infection for the tick vectors and sources of infection for possible sporadic outbreaks in the herd. To reduce the chances of sporadic outbreaks especially in susceptible adult cattle, smallholder farmers could vaccinate their animals using live or modified vaccines (Aubrey and Geale, 2010). It should be noted that vaccination does not prevent cattle from becoming persistently infected (Kocan et al., 2010); it does however, reduce the economic impact of the disease.

Higher infection rate of *A. marginale* in younger animals negatively affected the nutritional status (reduced BCS and PCV) of this group in the present study. Infection with *A. marginale* causes anorexia resulting in reduction of weight and condition (Aubrey and Geale, 2010; Kahn, 2006). *Anaplasma marginale*, replicates inside red blood cells causing increased extravascular haemolysis and reduction in the PCV (Kocan et al., 2010; Riond et al., 2008). The risk of infection with *A. marginale* was observed to be greater in poorly conditioned animals, thus showing an important link between nutrition and disease in cattle in the present study. Both cellular and humoral components of the immune system are required to combat infection of the blood borne haemoparasite. Adequate nutrition thus plays an important role in replenishing the molecules lost during the battle against infection. Improving the nutritional status especially in young cattle by providing supplementary feed can result in increased immunity (Marufu et al., 2010) and thus improved resistance of cattle in low input areas to *A. marginale* infection.

In the present study, genotype was observed to have no association with the molecular prevalence of *A. marginale* suggesting that there are no differences in innate immunity to anaplasmosis between the two genotypes. This finding contrasts the earlier report by Marufu et al. (2010) that genotype is associated with differences in sero-prevalence between Nguni and local crossbreds. Differences in tick loads, and hence transmission rates of the TBD, were cited as the reasons for genotype related differences in *A. marginale* prevalence in the earlier study, which was not the case in the current study. Bock et al. (1999) reported similarities in prevalence between *Bos indicus* and their crosses in Australia and attributed this to similarities in innate resistance to infection in the two genotypes. Mattioli et al. (2000) hypothesized that a more effective cellular immune response in addition to innate immunity leads to superior resistance to tick borne infections in indigenous cattle. It should be noted that all breeds of cattle may be at risk of severe disease if exposed to virulent *A. marginale* especially for the first time (Bock et al., 1999).

Nguni cattle tended to be more resilient to the adverse effects of infection with *A. marginale* than local crossbreds, despite the similar odds of infection in the two genotypes studied presently. The Nguni genotype managed to maintain BCS despite infection with *A. marginale* serving as testimony to the genotype’s possible superior resistance to anaplasmosis. The actual mechanism that confers resistance to *A. marginale* in indigenous Nguni cattle still remains unclear. Further investigations to elucidate the mechanisms of resistance to *A. marginale* infection in the Nguni genotype are required as these will improve knowledge on genotype related host resistance in indigenous cattle. The high tick infestation levels in cattle in small scale farming areas could result in direct losses such as tick worry, anaemia, damage to hides and skins of animals and tick toxicoses. To avoid direct losses caused by high tick infestations in cattle, small scale farmers could select for and breed cattle with shorter and smoother coats as they tend to be less susceptible to ticks (Marufu et al., 2011). The resultant reduced tick load could also play an important role in reducing challenge to cattle but maintaining endemic stability to *A. marginale* in cattle.

CONCLUSIONS

The molecular prevalence of *A. marginale* was moderate in the low input production system with cattle in the small scale farms having higher prevalence than those in the communal areas. A situation of endemic stability to bovine anaplasmosis was observed characterised by the absence of clinical disease despite high levels of infection in calves, and a high level of immunity in adult cattle. Animals were more likely to be infected with *A. marginale* if they were young, resident on small scale farms and in poor body condition. Nguni cattle were more resilient to anaplasmosis and suffered less severe losses from *A. marginale* infection than local crossbreds. Further elucidation of the genotype associated mechanisms of resistance to anaplasmosis in indigenous Nguni cattle is required.

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CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1: Molecular prevalence of *Anaplasma marginale* in Nguni and local crossbred cattle in the communal areas and small scale farms
Prevalence (%)

Management type	Nguni	Crossbreed	Overall	χ^2
Communal	27.3	35.7	30.6	0.6
Resettlement	85	86.5	85.7	0.8

Table 2: The odds ratio estimates, lower and upper confidence interval (CI) of an animal being infected by *Anaplasma marginale* in the smallholder areas

Infection with <i>A. marginale</i>	Point estimate	Lower CI	Upper CI
Management type	15.52	6.41	37.59
Tick infestation level	7.45	0.83	67.07
Body condition score	2.95	1.19	7.29
Age	0.56	0.36	0.88

Table 3: Least square mean body weight of Nguni and local crossbred cattle in small scale and communal areas

Production system	Genotype	Infected	Non-infected
Communal	Nguni	327.3 \pm 33.69	363.8 \pm 14.59
Small scale	Local crossbred	284.9 \pm 23.82 ^a	388.3 \pm 24.65 ^b
	Nguni	331.8 \pm 27.74	370.2 \pm 14.15
	Local crossbred	321.6 \pm 39.33 ^a	393.4 \pm 26.46 ^b

^{a, b, c} Means in the same row with different superscripts are significantly different at $P < 0.05$.

Table 4: Least square mean body condition scores of infected and non-infected Nguni and local crossbred cattle

Genotype	Nguni	Local crossbreed
Infected	2.68 \pm 0.133 ^c	2.24 \pm 0.113 ^a
Non-infected	2.74 \pm 0.156 ^c	2.39 \pm 0.085 ^b

^{a, b} Means with different superscripts are significantly different at $P < 0.05$.

Table 5: Least square mean packed cell volume of infected and non-infected young and old cattle

Age	Infected	Non-infected
Young (< 2 years)	2.35 \pm 0.137 ^a	2.58 \pm 0.147 ^b
Old (> 2 years)	2.69 \pm 0.146 ^b	2.79 \pm 0.188 ^c

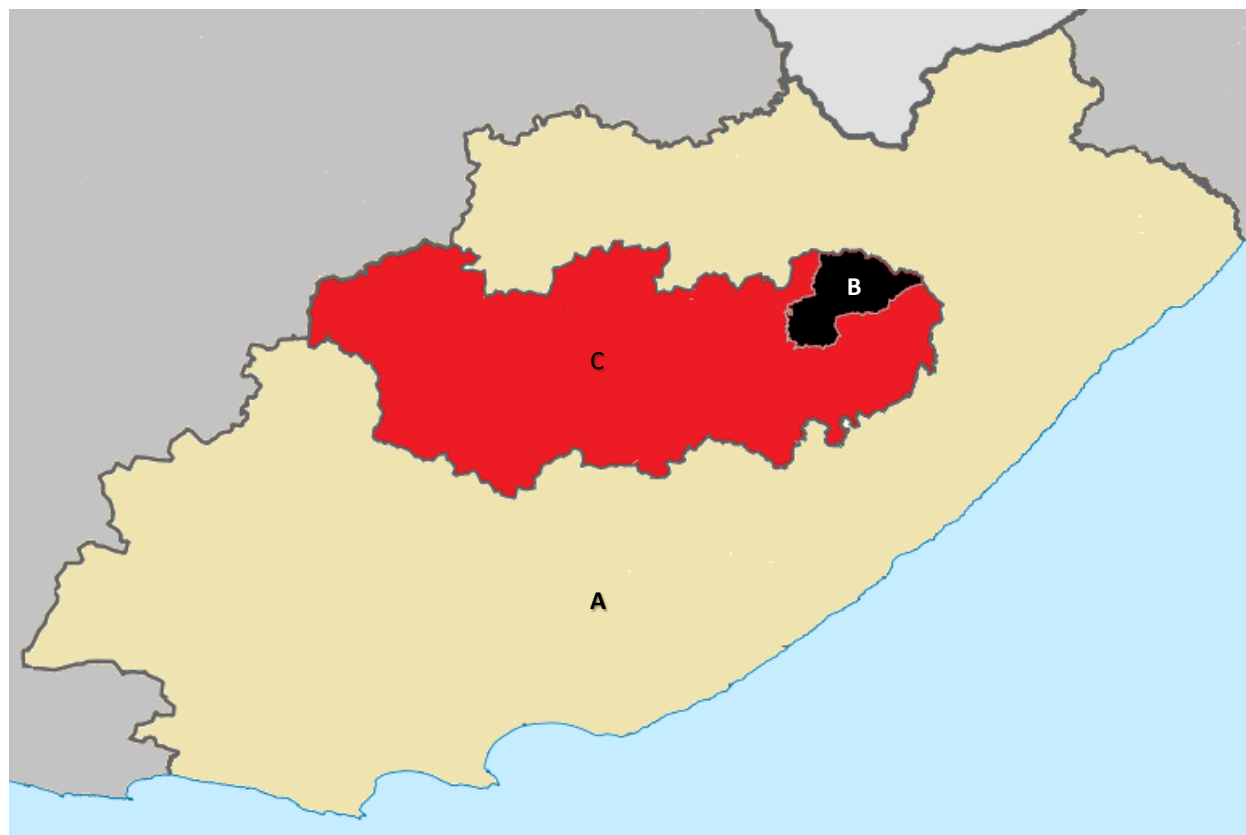


Figure 1. Map of the Eastern Cape Province (A) showing the study area Sakhisizwe Municipality (B) within the Chris Hani District Municipality (C)

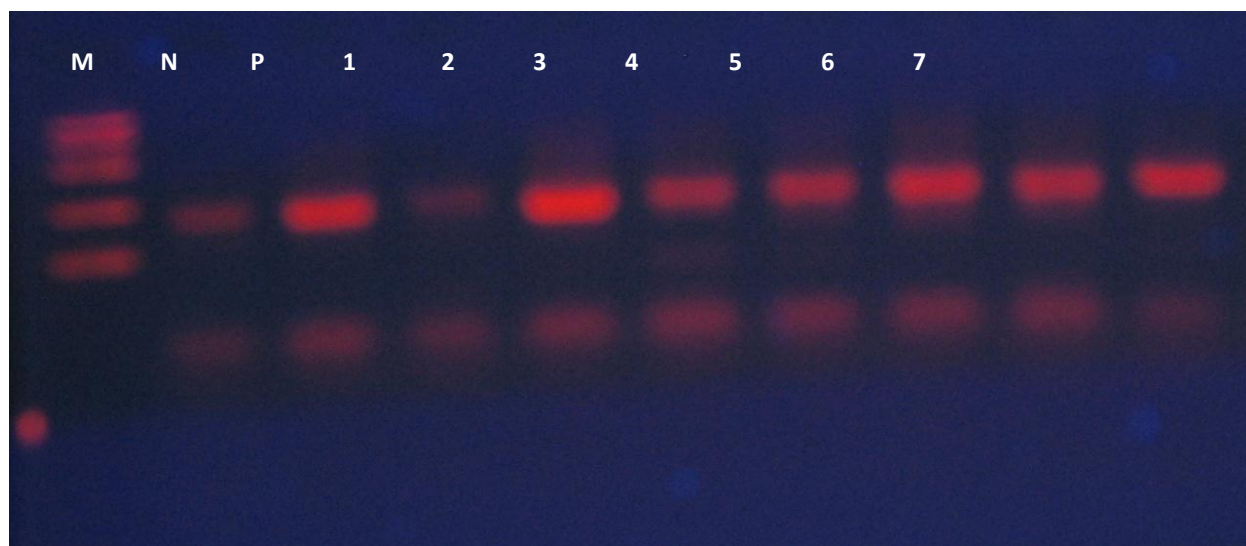


Figure 3: Photograph of the gel plate (A and B) showing some of the PCR products visualized under ultraviolet illumination. M: Molecular marker, N: Negative control, P: Positive control, 1-8: Test samples. P and Test sample 2 were strongly positive while samples 3-7 were weakly positive and N and Test sample 1 were negative.

INVASIVE NON-TYPHOIDAL SEROTYPES ISOLATED IN THE GREATER GAUTENG REGION IN 2011

Petty D. B.*, Henton M.M, Kidanemariam A., Rauff D., Thomas J. & Baloyi S.

ABSTRACT

Non-typhoidal *Salmonella* (NTS) data from four laboratories testing animal and feed samples as well as data from the National Institute for Communicable diseases of the National Health Laboratory Service representing human NTS isolates were compared and discussed. Although the most common NTS isolated from humans was *Salmonella enteritidis*, *S.typhimurium* was isolated from the majority of animal-related samples. Since the majority of human NTS isolates represent invasive HIV-associated disease, the predominance of *S. Enteritidis* may represent anthroponotic as well as zoonotic transmission, as previously shown in studies. However, the reluctance of farmers to have Group D NTS isolates typed in order to avoid the consequences of reporting a controlled disease should there be *S. Enteritidis*, is likely contributing to the observed discrepancy between predominant human and animal NTS isolates.

In Gauteng Province (GP), *S. anatum* is commonly isolated from animals, feed, water and human samples. Since this serogroup has not been shown to be important in anthroponotic transmission in the context of HIV-infection, it is extremely likely that the environment provides a significant source of this organism, so environmental (including water) testing of poultry farms should be encouraged, and the risk of transmission to and carriage of *S. anatomy* in workers borne in mind.

INTRODUCTION

Salmonella are gram negative, rod shaped, non-spore forming bacteria. There are over 2600 serotypes of *Salmonella*, many of which cause disease in both humans and animals. The antigenic formulae of serotypes are defined and maintained by the World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, according to the Kauffman- White scheme (Brenner,2000).. Currently, the genus *Salmonella* contains two species (*S. enterica* and *S. bongori*), each of which contains multiple serotypes. *S. enterica* is divided into six subspecies, and most serotypes infecting warmblooded species belong to *S. enterica* subsp. *enterica* (subspecies I).(Grimont, Grimont , & Bouvet P, 2000).

Within *S enterica* subsp *enterica*, serotypes are identified on the basis of antigenic formulae according to O (somatic) and H (flagellar) antigens, and the name usually refers to the geographic location where the serotype was first isolated (Brenner, Villar , Angulo, Tauxe, & Swaminathan, 2000). The genus and serotype, or group if applicable, will be used in this presentation to name the organism (Brenner, Villar , Angulo, Tauxe, & Swaminathan, 2000)(Forshell & Wierup, 2006).

While some *Salmonella* serotypes are host specific, the majority can affect multiple hosts. . NTS infection in humans is usually manifested as a localized enterocolitis, but in certain risk groups (including infants, immunosuppressed persons particularly those with HIV-infection, and the elderly) can result in systemic infection and serious complications that may be fatal. (Forshell & Wierup, 2006)(CDC, 2012).

An example of a *Salmonella* serotype which causes disease in the species that it is adapted to is *S. gallinarum* (, and this is considered less pathogenic to people (Forshell & Wierup, 2006); however, other serotypes often affect both animals and humans (including *S. typhimurium*, *S. enteritidis*, *S. hadar*, and *S..infantis*) (EU 2001/2003). The serotypes most commonly associated with human infections are *S. Typhimurium* and *S. Enteritidis*(Herikstad H, 2002)(Vieira, 2009). In most animal species, *Salmonella* establish an inapparent infection of variable duration that is a potential zoonosis. Under stress, *Salmonella* may cause disease in animals.

Animals infected after exposure to infected animals or feed, or due to a contaminated environment, excrete large numbers of *Salmonella* by faecal shedding. This can provide a source of contamination for other animals and the environment. Although the role of feed contamination with *Salmonella* in South Africa is not known, it has been shown to be important in other countries (Crump , Griffin , & Angulo , 2002). Faecal intestinal contamination of carcasses is the principal source of foodborne *Salmonella* infections in humans (Forshell & Wierup, 2006), human infections may also result from

*Contact Author- Gauteng Department of Agriculture and Rural Development

78 Eloff Street Glencairn

Johannesburg

2000

0833910686

Deryn.Petty@gauteng.gov.za

contaminated animal products like milk and eggs. Human and animal faecal contamination of vegetables or fruit may also become a source of foodborne infections (Forshell & Wierup, 2006).

In many parts of the world, there has been a dramatic increase in the incidence of NTS disease, while in others, the incidence has stabilised. In the EU alone, in 2009 there were 108614 cases of *Salmonella* (Scientific report of EFSA, 2011). The CDC estimates that about 400 persons per year die of acute salmonellosis in the USA alone (CDC, 2012).

MATERIALS AND METHODS

Four laboratories processing animal \pm environmental samples that are situated in Gauteng Province (GP) (Deltamune, Idexx, Faculty of Veterinary Science, University of Pretoria, and ARC-OVI bacteriology laboratory) were asked to submit records of all *Salmonella* isolates for 2011 from sites in GP and immediate environs. Although it was not possible in all cases to be certain of the site of sample collection, it is highly likely that these sampling sites were either in GP or immediate surrounds.

Different types of specimens were submitted for laboratory testing. These included faecal swabs, organs, intestinal contents, and in the case of poultry: boot covers and dust samples. The use of these environmental samples is commonly used to detect the presence of *Salmonella* infection in poultry flocks. Isolation of *Salmonella* in these types of environmental samples makes it highly likely that at least some of the birds in the flock will become infected as a result of the environmental contamination (Hutchinson, Moore, Sayers, Allen, & Davies, 2004).

Data for human cases was made available by the GERMS surveillance programme, National Institute for Communicable Diseases of the National Health Laboratory Service, and represents laboratory-confirmed invasive human NTS infections throughout the country.

RESULTS AND DISCUSSION

Of the 1 779 human NTS cases reported in South Africa for 2011, 763 cases (43%) were in GP. These isolates represented 74 serotypes of *Salmonella*.

It is likely that human-to-human (anthroponotic) transmission and environment-to-human transmission accounts for a high number of these cases. Most human cases are due to *S. enteritidis* and *S. typhimurium*, in keeping with global trends (EU 2001/2003)(Vieira, 2009).

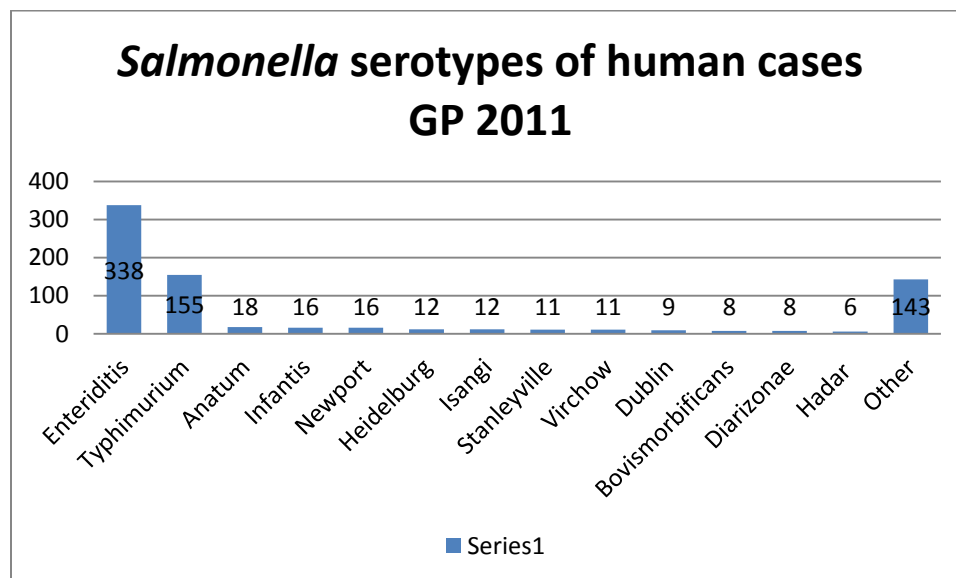


Figure 6: *Salmonella* serotypes of human cases, Gauteng Province, 2011 (GERMS 2011)

236 cases of NTS were identified by the four GP laboratories testing animal \pm environmental samples. Based on the sample submission date, it is not possible to detect any seasonal trends in the occurrence of positive samples. The majority of isolates were related to poultry, which likely reflects the higher rate of submission of samples for that farming sector.

Relatively few isolates were from pigs, which is probably related to the low rate of sample submission by pig farmers and abattoirs. It is estimated that worldwide, 20% of human salmonellosis is related to the consumption of pork (EFSA Scientific Committee, 2010). This is an area therefore that should be given some attention in South Africa.

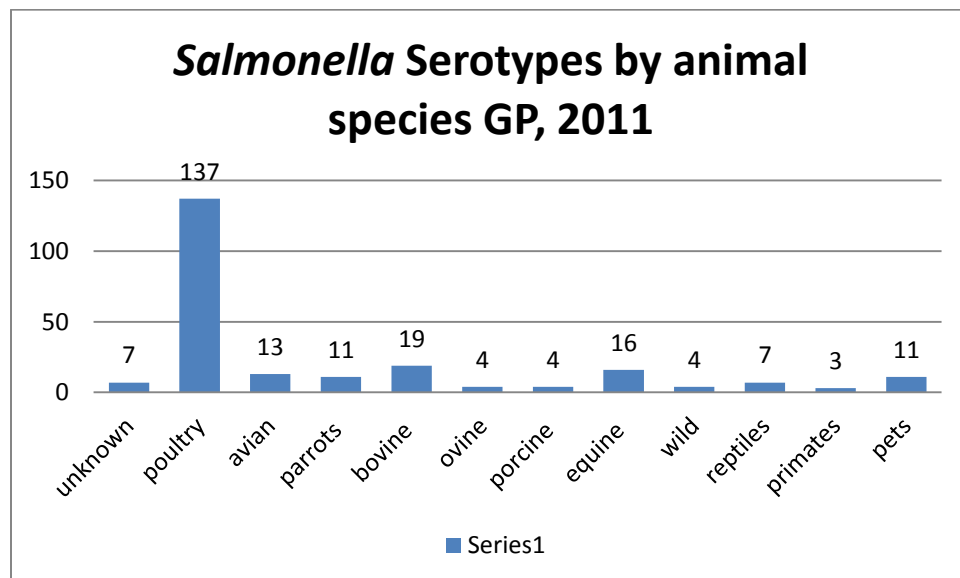


Figure 7: The number of Salmonella isolates by animal species in the greater Gauteng area, 2011

A high percentage of these samples were either typed only to serogroup or were not typed. This is probably due to financial constraints, but does also reflect unwillingness on the part of farmers to report *S. Enteritidis*, which is a controlled disease in South Africa.

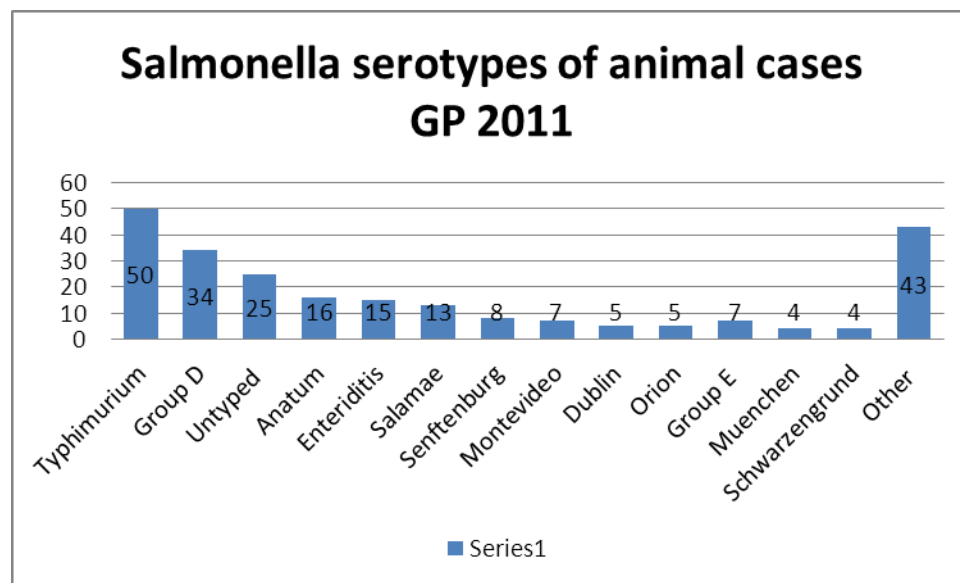


Figure 8: Salmonella serotypes of animal cases, GP, 2011

S. anatum is an uncommon cause of human NTS disease, but in GP this serotype accounts for 2.4% (18/763) of human NTS cases. Interestingly, *S. anatum* is frequently isolated from healthy poultry workers, poultry feed, poultry samples and water in the province.

CONCLUSION

Many of the same NTS serotypes cause infections in both humans and animals. Of isolates from animal cases characterized to serotype level, *S. typhimurium* was more common than *S. enteritidis*. A large number of the group D isolates from animal cases which were not typed further are likely to be *S. Enteritidis*, and therefore *S. Enteritidis* is certainly under-reported in animals. Of interest is the higher proportion of *S. anatum* human cases in GP as opposed to the rest of the country, in concert with *S. anatum* being detected in feed, poultry workers and water samples in the province. This would suggest that there is an environmental source of *S. anatum* which is localized to GP and is resulting in human infections. Farmers are therefore advised to perform environmental testing, and the risk of infection to farm workers must be borne in mind.

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ARC Onderstepoort Veterinary Research Institute, bacteriology section 100 Old Soutpan Rd, Onderstepoort,

University of Pretoria, Faculty of Veterinary Science, Tropical Diseases, Section of Bacteriology.

IDEXX Laboratories (Pty) Ltd. Woodmead Office Park 19b Morris Street East

Woodmead extension 1 2146 Sandton, Johannesburg, South Africa

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THE POULTRY DISEASE MANAGEMENT AGENCY (PDMA)

Nkuna, C*.

The Poultry Disease Management (PDMA) agency was conceived by Industry in conjunction with the University of Pretoria in 2009 with the aim of providing the South African poultry sector with cost effective disease control. The PDMA is financed through the levy paid by the producers. The South African poultry sector is important not only to the direct stakeholders, but also to the nation as a whole. As an employer of over 70 000 people, as well as generating over R47 billion in annual turnover, it is easy to see why the survival of the industry is critical to the government's job and food security agendas.

The decentralisation of the State veterinary services has diluted the National Agricultural Department's ability to effectively control diseases because the provinces have the autonomy on disease control measures. Those provinces that have good systems manage to effect disease controls and are able to stamp out disease outbreaks before they spread. However, those with inefficient systems have in the recent past struggled to control disease outbreaks. Since diseases know no boundaries, the disease control efforts are as strong as their weakest link.

After a great deal of planning, debates and discussions, the sector was left in no doubt as to the need for such an agency in order to protect the national flock and ensuring that the industry continues to make its significant contribution to the nation's zero hunger ambitions. The agency has very clear objectives which are as follows:

1. Engage national and local government on the issues of disease control in the South African poultry industry.
2. Make use of the database of poultry farms in South Africa to assist DAFF with monitoring of notifiable disease such as Avian Influenza, Salmonella and Newcastle Disease, while using it to develop monitoring programmes for important disease such as Infectious Bronchitis.
3. Appoint or designate veterinarians with expertise in poultry diseases in each Province who would be available to assist state veterinarians in the event of disease outbreaks in commercial, smallholder and subsistence poultry in those provinces.
4. Investigate the role of the PDMA in training state veterinarians and/or Animal Health Technicians so as to improve the service delivered by the state in the event of disease outbreaks on poultry farms.
5. Consider developing a residue monitoring programme for poultry products nationally, or at least a database of residue monitoring data which is available.
6. Deliver improved technical and veterinary support to small scale poultry farmers so that they can achieve greater production success in collaboration with state veterinary services or through the PDMA's own initiatives.
7. Collaborate with the ostrich industry.

It is clear from the above stated objectives, that the PDMA will have strong ties with the government at both national and provincial levels to perform its duties optimally. The agency will be the connector of all stakeholders involved in poultry disease surveillance, monitoring, control and management. It is envisaged that the structure of the PDMA's relationships with the other sector stakeholders will be as outlined in *figure 1* below.

Figure 1: Poultry Disease Management Agency stakeholder relations outline.

