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PAPERS

AN APPROACH FOR THE FIT AND COMPARISON OF DIFFERENT BAYESIAN MODELS FOR THE VALIDATION OF DIAGNOSTIC TESTS

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SUMMARY

We describe a stepwise approach for the Bayesian validation of diagnostic tests in absence of a gold standard. This stepwise approach, based on the Deviance Information Criterion (DIC), allows us to compare the simplest model (conditional independence model) with gradually more complex models allowing for conditional covariance between the sensitivities and specificities of one or two pairs of tests. Data are from a survey for *Trypanosoma evansi* in dromedary camels in Niger. All 729 animals were tested with a parasite detection test and three antibody detection tests. The model which allows a conditional dependence between the indirect agglutination test and the ELISA appeared to be the better model. This can be expected biologically since these two tests are based on the same purified antigen.

INTRODUCTION

Bayesian inference using Monte Carlo Markov Chain (MCMC) techniques for the validation of diagnostic tests in absence of a gold standard gain more and more popularity. Most models however are still based on the assumption of conditional independence between the different tests and assessment of the goodness of fit of the models is rarely executed. Relaxing the conditional independence assumption requires more diagnostic tests in the experiment setup to avoid overparameterization or the availability of reliable prior information on one or more parameters. In addition it is essential to verify whether the new, expanded model is in fact a model that better explains the observed data.

We compared 14 models for the validation of four diagnostic tests for *Trypanosoma evansi* causing Surra in dromedary camels (a widespread parasitological disease responsible for important economical losses) starting with the simplest model under conditional independence and gradually increasing the complexity by including conditional covariances between test pairs. Model comparison was based on the DIC (Spiegelhalter *et al*., 2002) a Bayesian version of the Akaike Information Criterion (AIC, Atkinson, 1980). This stepwise approach of adding more parameters followed by model fit and comparison allowed the selection of the simplest model which explained the data the best.

MATERIALS AND METHODS

Sampling and diagnostic tests

Four diagnostic tests were conducted on all 729 samples obtained from a survey in Niger. A parasite detection test, the Mini Haematocrite Centrifugation Technique (MHCT), was conducted in the field. On the serum three serological tests (Verloo *et al*., 2000) were conducted. The CATT/*T.evansi* a direct agglutination test using freeze dried Coomassie stained whole trypanosomes of the RoTat 1.2 Variable Antigenic Type (VAT) (Bajyana Songa *et al*., 1988; Verloo *et al*. 2001); the LATEX/*T.evansi* an indirect agglutination test detecting agglutinating antibodies against the purified RoTat 1.2 Variable Surface Glycoprotein (VSG) bound to latex particles and an Enzyme Linked Immune Sorbant Assay (ELISA/*T.evansi*)) test detecting different antibodies against the RoTat 1.2 VSG.

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Statistical models

As summarized in Table 1 a total of 14 latent class models (Walter *et al.*, 1988) were fitted (multinomial likelihood). All priors were uninformative beta(1,1) distributions except for the priors on the conditional covariances which were uniform(0,1) with an upper limit min(se1,se2)-se1*se2 for those between two sensitivities and min(sp1,sp2)-sp1*sp2 for the covariance between two specificities (Vacek, 1985). The conditional covariance was constrained to be positive because negative dependence is biologically unlikely to exist (Vacek, 1985; Gardner *et al.*, 2000).

Posterior densities of all parameters were obtained by Gibbs sampling using WinBugs 1.4 software (Gilks *et al*.,1994). Three parallel sequences with different starting values were run for each model and convergence was monitored with the Gelman-Rubin statistic (Gelman *et al*.,1995). Once this statistic converged to 1 for all observed parameters (including the deviance), the burn in period was determined. After burn in all models ran for another 10,000 iterations. Goodness of fit of the models was assessed by visually inspecting the expected and observed data and by calculating posterior predictive p values (Bayes p) (Gelman *et al*.,1995) which is defined as the probability that the replicated data could be more extreme than the observed data, measured (in this case) with the $\chi 2$ discrepancy quantity (Splus, Combinat library). Bayes p values with extreme tail-area values correspond to major failures of the model.

Subsequent models were compared by monitoring the DIC after convergence of the deviance (a lower DIC value corresponds with a "better" model). The DIC is composed of a Bayesian measure for fit or adequacy (the posterior mean deviance) penalized with an additional complexity term pD being the difference between the posterior mean of the deviance and the deviance at the posterior mean of the parameters. We refer to Spiegelhalter *et al*. (2002) for more details on the DIC. All statistics of the posterior distributions (mean, 2.5^{th}, 50^{th} and 97.5^{th} percentiles) were rounded according to their Monte Carlo error.

RESULTS

As summarized in table 1 all models fitted the data according to the posterior predictive p values which showed no extreme values. However it was striking that models with Bayes p values leading away from 0.5 showed increasingly more discrepancies between the observed values and the distribution of the expected values (mostly in cells with a low or zero number of observations, graphs not shown). Note that for the model 5,6 and 9,10 we did not calculate a Bayes p value but as they were simplifications of the models 4 and 8 and the deviance was similar to those models the fit was also expected to be adequate. According to the DIC, model 8, which allows the LATEX and ELISA test to be conditional dependent for both sensitivity and specificity was found the better model followed close by model 4. Simplification of that model by adding the covariance between sensitivities alone (model 9) or specificities alone (model 10) did not improve the DIC (worse deviance and only a slight better model complexity score pD). Model 12 was obtained by combining models 4 and 8. Not surprisingly this model scored a very good DIC but it was not superior to model 8. The conditional independent model (1) staid a better candidate than lot of other models for which a covariance was placed between the wrong pairs of tests.

Summary statistics of the posterior distributions of the parameters in model 8 are shown in Table 2.

DISCUSSION

We agree with Box (1976) saying that that all statistical models are wrong but some are useful. Therefore model fitting, comparison and selection is crucial to statistical analysis but as stated by Spiegelhalter *et al.* (2002) an overformal approach to model 'selection' is inappropriate since so many other features of a model, e.g. the robustness of its conclusions and its inherent plausibility, should be taken into account before using it as a basis for reporting inferences. We believe that in

this case the better model (8) is also the biologically plausible model leading to biologically acceptable parameter estimates (data not shown) but of course there are other (non tested) models and other ways to model the conditional dependencies of diagnostic tests.

For this paper we did not try to model conditional dependencies between three or more tests. This because of the inherent difficulty in interpreting those interactions and the necessity on inclusion of prior information to fight the overparametrisation. Next to this is the conditional covariance as defined by Vacek (1985) not straightforward for modelling conditional dependencies between three or more tests and alternative parametrisations have to be used. Modelling all possible interactions between the tests however is statistical utopia. There is even no setup possible for such an analysis as adding diagnostic tests to the setup increases exponentially the lack of degrees of freedom.

Remarks can be made on the fact why these models where not worked out in a frequentist framework since they are all identifiable models and we did not gave any shape to the prior distributions of the parameters. We are however convinced that the flexibility and the ease of simulation from the posterior distributions (leading to distributions of other parameters of interest as predictive values, diagnostic accuracy parameters of test combinations, sample size calculations, etc.) is a major advantage of MCMC next to the fact that normal distribution approximations, in the frequentist framework commonly employed to derive confidence intervals around unknown parameters from estimated standard errors, are not required.

A lot of discussion is going on the use of the DIC as a Bayesian model comparison tool. One of the aspects is the pD as a measure for model complexity while in other information criteria like the widely used AIC the numbers of parameters to be estimated are a measure for model complexity. Using the pD however will allow the model complexity to be related to the data and the prior information put in the model which is very reasonable in the Bayesian context (e.g. penalization for adding 1 conditional covariance between two test sensitivities is restricted because of the constraint put on the parameter).

Table 1. Overview of Bayes p values, number of parameters to be estimated, deviance, pD (model complexity) and DIC for the different models

No	Covariance between	Bayes p	Param	Deviance	pD	DIC
	NONE					
1*	(CI model)	0.36	9	66.396	7.419	73.815
	ONE PAIR OF TESTS					
2	CATT-LATEX	0.36	11	66.287	7.668	73.955
3	CATT-ELISA	0.26	11	68.469	7.803	76.272
4*	MHCT-CATT	0.52	11	64.039	8.537	72.576
5	Se		10	66.108	7.768	73.876
6*	Sp		10	64.251	8.150	72.402
7	MHCT-LATEX	0.27	11	67.901	7.751	75.652
8*	LATEX-ELISA	0.57	11	63.398	8.219	71.617
9	Se		10	65.543	8.104	73.647
10	Sp		10	67.746	7.886	75.632
11	MHCT-ELISA	0.32	11	66.914	8.496	75.411
	TWO PAIRS OF TESTS					
12*	MHCT-CATT & LATEX-ELISA	0.51	13	63.779	8.556	72.336
13	ELISA-MHCT & CATT-LATEX	0.31	13	67.346	8.117	75.463
14	MHCT-LATEX & ELISA-CATT	0.18	13	70.114	8.072	78.187

Table 2. Summary statistics posterior distributions of model 8

Parameter	Mean	2.5th perc	50th perc	97.5th perc
Prevalence	0.5615	0.5233	0.5615	0.5987
Se MHCT	0.2548	0.2135	0.2545	0.2979
Se CATT	0.9756	0.9529	0.9762	0.9941
Se LATEX	0.9667	0.9433	0.9675	0.9854
Se ELISA	0.7856	0.744	0.7859	0.8248
Sp MHCT	0.9904	0.977	0.9914	0.9981
Sp CATT	0.9827	0.9577	0.9841	0.999
Sp LATEX	0.9833	0.9554	0.9855	0.9993
Sp ELISA	0.9798	0.9528	0.9817	0.9965

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ASSESSMENT OF POST TEST PROBABILITIES OF MULTIPLE TESTS IN THE ABSENCE OF A GOLD STANDARD IN ORDER TO SELECT APPROPRIATE TEST COMBINATIONS AND DECISION RULES

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SUMMARY

We describe the Bayesian validation of four antibody detection tests in absence of a gold standard. The aim of the study was to choose the best test combination to assess seronegativity of Belgian cattle against Bovine herpes virus 1. Four candidate ELISA tests were selected and executed on 920 field sera. Sensitivity, specificity and predictive values of all tests and different test combinations were assessed. Based on these results the best test combination and decision rule was chosen.

INTRODUCTION

EU directives for import and export of cattle are based on the fact that animals should be seronegative against Bovine herpes virus 1. Several antibody ELISAs are available but discrepancy between test results is frequent and questions raise on how to intepret these results and on which ELISAs to use in a sequential multiple test set up.

The Belgian reference lab selected two candidate blocking ELISAs and two indirect ELISAs and decided, in order to avoid dependence conditional on the seropositive status that multiple tests should be based on one blocking ELISA and one indirect ELISA. Guiding the decision on which blocking and which indirect ELISAs to choose and how to interpret the test results was the primary target of this study.

We used Bayesian inference by Monte Carlo Markov Chain (MCMC) techniques for the validation of the four candidate antibody ELISAs in absence of a gold standard. Pair wise dependence on the seropositive status was modelled and different biologically plausible models were fitted and compared. Tests an test combinations were evaluated according to their diagnostic accuracy (sensitivity and specificity) and their post test probabilities (predictive values).

MATERIALS AND METHODS

A total of 920 field sera were all, within the same laboratory, analyzed with four antibody detection ELISAs (2 glycoprotein B blocking ELISAs (T1 and T2) and 2 indirect ELISAs (T3 and T4)).

The Bayesian statistical approach and the model fitting and comparison are described in detail by Verloo & Büscher (2004). We compared different models using the deviance information criterion (DIC) (Spiegelhalter *et al.*, 2002) starting with the simplest model under conditional independence and gradually increased the complexity by including conditional covariances between test pairs. All priors were uninformative beta(1,1) distributions except for the priors on the conditional covariances which were uniform(0,1) with an upper limit min(se1,se2)-se1*se2 for those between two sensitivities and min(sp1,sp2)-sp1*sp2 for the covariance between two specificities (Vacek, 1985).

For different test combinations the sensitivity and specificity of a parallel (OR, believe the positive) interpretation scheme and a serial (AND, believe the negative) was calculated and corrected for conditional dependence according to Gardner (2000). We used the notations sep_{T1T2} (spp_{T1T2}) for the sensitivity (specificity) of a parallel, and ses_{T1T2} (sps_{T1T2}) for the sensitivity (specificity) of a serial interpretation scheme of, in this case, T1 and T2.

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Post test probabilities (PPV and NPV) were calculated using Bayes theorem. Inclusion of conditional covariances in the post test probabilities of multiple tests was as described by Gardner (2000). We used the notation $PPVpar_{T1T2}$ for the positive predictive value of a parallel interpretation scheme and $NPVser_{T1T2}$ which the negative predictive value of a serial interpretation scheme. Logically, the negative predictive value of a parallel interpretation scheme is $NPVpar_{T1T2}$ = 1-Pr(D+ | T1-,T2-) while the positive predictive value of a serial interpretation scheme $PPVser_{T1T2}$ = Pr(D+ | T1+,T2+).

When comparing tests or test combinations at the level of the diagnostic accuracy (se and sp) or at the level of the post test probabilities (PPV and NPV) we used se+sp as an overall measure of accuracy and PPV+NPV as a measure for post test knowledge.

Comparison of posterior distributions or linear combinations of posterior densities was done by random drawings from these distributions. To determine the confidence (a value between 0 and 1) that one parameter was higher than another parameter we calculated the proportion of draws which fulfilled that criterion.

RESULTS

According to the DIC the model which allowed a conditional dependence between the sensitivities and specificities of the two indirect ELISAs and two blocking ELISAs respectively, appeared to be the simplest model which explained the data the best (DIC=58.25 compared to a DIC of 150,73 for the conditional independent model; no further details on model comparison shown). Table 1 summarizes the posterior densities of the parameters of this model.

Comparison of the se+sp of the two blocking ELISAs and the two indirect ELISAs resulted in a 0.99 confidence that test 1 was the better one of the two blockings and a 0.64 confidence that T4 was the better one of the two indirect ELISAs. This was confirmed by the post test probabilities where the Pr(PPV1+NPV1>PPV2+NPV2) results in a 0.905 confidence and Pr(PPV4+NPV4>PPV3+NPV3) = 0.89

For above reasons it was chosen to continue the analysis of the multiple test setups with T1 as the preferred blocking ELISA and T3 or T4 as the indirect ELISAs.

When comparing Pr(D+ | T1+,T3+) and Pr(D+ | T1+,T4+) there is a 0.804 confidence in having a higher post test probability for the T1T4 combination while for the Pr(D- | T1-,T3-) and Pr(D- | T1-,T4-) no obvious difference can be found (0.523 confidence that the T1T4 combination is higher).

In addition, for a serial interpretation scheme the Pr(ses13+sps13<ses14+sps14)= 0.655 while for the PPVser + NPVser this is approximately 0.5. For a parallel interpretation scheme the Pr (sep13+spp13<sep14+spp14) ≈ 0.5 while for the sum of the PPVpar and NPVpar there is a 0.973 confidence that the T1T4 combination is the better one. This is completely caused by the difference in PPVpar although the difference is minimal (mean=median = 0.01 with a symmetric 95% credible interval of [-0.001 ; 0.025]).

As can be expected, for both T1T3 and T1T4 the PPVser is higher than the PPVpar while the NPVpar is higher then the NPVser.

Table 1. Posterior densities for the best fitting model

Parameter	**mean**	**2.5 %**	**median**	**97.5 %**
Prevalence	0.2994	0.2657	0.2994	0.3339
$sensitivity_{T1}$	0.9953	0.9828	0.9968	0.9999
$sensitivity_{T2}$	0.9869	0.97	0.9881	0.997
$sensitivity_{T3}$	0.9206	0.8596	0.9216	0.9731
$sensitivity_{T4}$	0.9203	0.8594	0.9213	0.973
$specificity_{T1}$	0.9722	0.9449	0.973	0.9938
$specificity_{T2}$	0.9438	0.9148	0.9446	0.9684
$specificity_{T3}$	0.9932	0.9855	0.9937	0.9981
$specificity_{T4}$	0.9984	0.9941	0.9989	0.9999
PPV1	0.9382	0.8774	0.9402	0.9865
PPV2	0.8821	0.82	0.8838	0.9346
PPV3	0.983	0.9639	0.9843	0.9952
PPV4	0.996	0.9852	0.9972	0.9999
NPV1	0.998	0.9925	0.9986	0.9999
NPV2	0.9941	0.9864	0.9947	0.9986
NPV3	0.9666	0.9389	0.9673	0.9894
NPV4	0.9667	0.9391	0.9674	0.9893
sep13	0.9996	0.9985	0.9998	1.0
sep14	0.9996	0.9984	0.9998	1.0
ses13	0.9163	0.8544	0.9174	0.9696
ses14	0.916	0.8547	0.9169	0.9695
spp13	0.9656	0.9376	0.9663	0.9884
spp14	0.9706	0.9434	0.9714	0.9926
sps13	0.9998	0.9995	0.9998	1.0
sps14	1.0	0.9998	1.0	1.0
$Pr(D+ \mid T1+,T3+)$	0.9995	0.9986	0.9996	0.9999
$Pr(D+ \mid T1+,T3-)$	0.5511	0.1875	0.5569	0.8976
$Pr(D+ \mid T1-,T3+)$	0.2152	0.006818	0.1717	0.6428
$Pr(D+ \mid T1-,T3-)$	1.664E-4	3.474E-6	1.008E-4	7.114E-4
$PPVpar_{T1T3}$	0.9251	0.8634	0.927	0.975
$NPVser_{T1T3}$	0.9651	0.9372	0.9659	0.988
$Pr(D+ \mid T1+,T4+)$	0.9999	0.9995	0.9999	1.0
$Pr(D+ \mid T1+,T4-)$	0.5509	0.1879	0.5545	0.8978
$Pr(D+ \mid T1-,T4+)$	0.5268	0.03131	0.54	0.9733
$Pr(D+ \mid T1-,T4-)$	1.675E-4	3.471E-6	1.01E-4	7.143E-4
$PPVpar_{T1T4}$	0.9353	0.8745	0.9373	0.984
$NPVser_{T1T4}$	0.965	0.937	0.9657	0.9881

DISCUSSION

It is concluded that the both T1T3 and T1T4 combination in a parallel interpretation is the best option for the aim of detecting seronegative animals with more or less equal and very high NPVpar.

The T1T4 combination however had a slightly higher PPVpar giving a better all-round post test knowledge (PPVpar+NPVpar). Although this is not directly visible in the summary statistics of both parameters as both credible intervals substantially overlap there is a big confidence of having a higher $PPVpar_{T1T4}$ then a $PPVpar_{T1T3}$. This may seem odd because the mean difference is only 0.01 but is due to the fact that the distributions are heavily skewed (not shown). We should keep in mind however that, if the aim is to maximize the PPV, one would not choose for a parallel interpretation scheme but use a serial instead.

Although one could say that it is not necessary to use any interpretation scheme because we know the PPV and NPV of every test combination it is a fact that in real life the test will be used in sequence and testing will be stopped if the first test is negative (believe the negative; serial) or vice versa when it is positive (believe the positive, parallel) for financial reasons.

Comparison of diagnostic accuracy between different tests based on one cut-off value does not fully represent the diagnostic power of a test (Greiner *et al.*, 2000) and may even be misleading. As an alternative ROC analysis is suggested. In this setup however cut-off values are fixed in the standard operating protocol and cannot be changed. In addition we do not recommended to use this "no gold standard" latent class approach to construct ROC curves.

For this study the latent variable D will rather define a sort of seropositive status of the animal instead of the disease status therefore influencing the conditional probabilities se and sp and the post test probabilities. This may be a major comment as we don't really assess here the diagnostic capacities of the test. We are however convinced that, as the regulations are based on the seronegative status of the animal that the estimates from this model are valid in this framework.

Trying to estimate the "real" diagnostic capacities of the test would require the modelling of higher level dependencies. However, modelling these higher interactions lead inevitably to overparametrisation of the model making it highly prior driven. Next to this, even in a setup with an infinite number of diagnostic tests it is not possible to model all possible dependencies as the lack of df increases exponentially with the addition of tests. Modelling conditional dependencies and/or checking for it by, for example, looking at the conditional log odds ratio (not shown) will therefore always be restricted. This leads to the conclusion that, as stated by Pepe (2003), that the definition of the latent variable D and as a consequence the conditional probabilities based on that parameter will always be dependent on the tests included in the setup which makes it rather subjective. On the other hand, the assumption of a gold standard may even be worse as there is no real definition for D, which makes it a rather artificial way to solve the problem. Keeping this in mind it is reasonable that one should distinguish between D which is unobservable and the diagnosis which is observable (Kraemer (1992).

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BOVINE TUBERCULOSIS IN SOUTHERN AFRICA: IS IT TIME FOR AN INTEGRATED APPROACH TO ZOONOTIC DISEASE POLICY?

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INTRODUCTION

In 2001, Taylor et al. reported that ~75% of pathogens have a zoonotic capacity. Other studies showing similar results (Dasak et al. 2001, Cleaveland et al. 2001) confirm that it is imperative to understand the fundamental dynamics of infectious diseases in order to mitigate the impacts on public health, wildlife, and livestock economies (Cleaveland et al. 2001). Collaboration between multi-disciplinary scientists, including veterinarians, medical doctors, epidemiologists, economists and sociologists, is therefore requisite in order for the breadth of disease research to mirror the vast range of illness caused by multi-species pathogens.

THE HISTORY OF COLLABORATIVE RESEARCH

The history of collaborative research dates back to France, when in 1862, the first chair in comparative medicine (designed to link animals and human health disciplines) was established (Wilkinson, 1992). Synergistic research has a respected history, which contributed to the discovery of cattle fever in 1893, yellow fever (Wilkinson, 1992) and provided the basis for Nobel Prize winning work on immune system responses to viral infections (Zinkernagel & Doherty, 1974. Prize winners in 1996). However, in contrast to the growing need for multi-disciplinary infectious disease research, the collaborative aspects of the required sciences remain fragmented. Responsibilities within disease surveillance programmes may be split across national and state level departments, across agencies (agricultural, health, environmental) and local and international borders. This results in confusion as to who is responsible for monitoring disease and to whom findings should be reported. In addition, funding bodies have yet to recognise the importance of promoting interdisciplinary research (Zerhouni, 2003) with only 4.7% of National Institutes of Health (NIH) grants being awarded to veterinarian lead research in 2001 (NAS, 2004).

Nonetheless, a general resurgence in the philosophies of inter-disciplinary science has yielded promising results in Africa by the implementation ‘One Medicine’ (Schwabe, 1984) and ‘One Health’ (Kock, 2005) philosophies. These principles combine human, animal and ecosystem health, which enable greater service capabilities than those offered by each discipline alone (Alliance for Health Policy and Systems Research, 2004). This has resulted in solution orientated institutional reform, designed to encourage successful cross-disciplinary partnerships (Zinsstag et al. 2006).

INTEGRATED HEALTH CARE: EXAMPLES FROM AFRICA

Zoonotic disease often breeds confusion amongst specialists who recognise the need for research but lack the broad knowledge required to investigate the emerging patterns from an holistic perspective (Zinsstag et al. 2006). Surveys conducted by Grant and Olsen (1999) revealed that physicians were “generally not comfortable” discussing the role of animals in the transmission of zoonoses, and would prefer that veterinarians play a role (Kahn, 2006). However, medical patients did not view veterinarians as a source of information for human health (Grant and Olsen, 1999). Indeed, state veterinarians in the USA were also unsure if they had a role to play in reporting potential zoonotic diseases, with only 8% responding positively to their perceived involvement (Kahn, 2006).

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Encouragingly, examples of successful collaborative health services are available. These include not only short-term collaborations during humanitarian crisis, droughts and civil wars, (e.g. International Red Cross vaccination campaigns using veterinary vehicles, Schelling et al. 2005b), but also longer-term sustained efforts in low-income rural areas. These have been particularly effective in areas with limited health services. As over 80% of African human and animal populations reside in areas with no health care (Cosivi et al. 1998), collaborative efforts are now encouraged by the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) in order to maximise resources and widen health care distribution (Meslin, 1996).

Prior to the integration of human and animal health programmes in Chad, no children had been fully immunised, although community leaders had requested vaccinations against measles and whooping cough (Daoud et al. 2000). However, successful compulsory animal health campaigns had resulted in 75% of cattle and camels being vaccinated by veterinary teams (Schelling et al. 2005b). A joint vaccination programme was established as a result of consultation between the ministries of health and livestock production and nomadic communities, which identified health as a top priority for regional livestock and livelihood sustainability. Simultaneous livestock and human vaccinations were conducted between 2000 and 2004. With the exception of species-specific vaccines, all resources were shared between local health and veterinary staff. This prevented the creation of parallel structures, and reduced the associated costs by 15% (Bechir et al. 2004). More importantly, 103,521 livestock were vaccinated, 4,022 children younger than five were fully immunised and 6,284 women received at least partial tetanus immunisation (Schelling et al. 2005b).

The pre-vaccination education of communities by medical and veterinary staff encouraged the spontaneous community take-up of services and reduced the 'loss to follow-up' usually experienced. The inclusion of stakeholders and communities during the planning stages of the campaigns increased general disease awareness and amplified the 'perceived usefulness' of the programme (Schelling et al. 2005b). In turn, the provision of health services via the inclusion of local care workers encouraged communities to take responsibility and ownership of their health needs. The final outcome was the creation of a national 'Extended Programme on Immunisation' to continue the successful collaborations between veterinary and medical services and communities in Chad.

Similar programmes have also been successful outside Africa. Indeed Italy has recently established a joint ministry for human and animal health (Schelling, 2005). In both cases, incremental improvements in health care have been achieved by the inclusion of different health disciplines and trusted local care workers (Schelling et al. 2005b). Location is therefore irrelevant for the success of the 'One Health' strategy, providing all partners consider the involvement of all disciplines to be equally important. Combined strategies help to foster communication between human and animal health professionals, which in turn facilitates more effective disease monitoring and faster identification of opportunistic and zoonotic infection (Schelling et al. 2005b).

These principles can be applied to a range of health issues in southern Africa. If correctly administered, collaborative programmes should reduce costs for both the financially overstretched and resource limited veterinary and medical sectors. Ultimately, the resulting improved effectiveness of testing and vaccination campaigns in rural areas would improve health care where it is needed the most. However, for the inter-sectoral approach to be sustainable and credible both within and across countries, joint planning, management and coordination of policies is required (Majok and Schwabe, 1996). Examples where this level of organisation should be introduced immediately include the monitoring of disease across international borders in the Greater Limpopo Transfrontier Conservation Area (crossing South Africa, Zimbabwe and Mozambique; Osofsky, 2005) and the Economic Community of West African States (crossing 15 countries in West Africa; Zinsstag et al. 2006). Both these projects will also require complementary and alternative forms of ecosystem management to link in with health services and ensure sustainable development and conservation in South Africa (Forget and Lebel, 2001).

HOW THIS APPLIES TO ZOONOTIC DISEASE IN AFRICA: AN EXAMPLE OF BTB

Zoonotic disease is prevalent throughout Africa, and is the source of a number of high profile diseases, including HIV and Ebola (Blancou et al. 2005). The combination of infectious diseases including HIV, TB and malaria places a huge burden on low-income countries in southern Africa. This increases the vulnerability of human and animal populations to infection, particularly in areas with poor disease control and limited health care services.

Bovine tuberculosis (BTB) is endemic throughout Africa and infects a range of species including valuable and endangered wildlife, agricultural and domestic animals and humans (Grange, 2001, Thoen et al. 1995). Introduced to Africa via imported European cattle in the 18th Century, the resulting decrease in agricultural productivity led to the implementation of a National TB Control and Eradication Scheme by 1969 (Michel, 2002). However, little is known on the impact of this scheme outside the commercial beef and dairy sector. Despite over 80% of Africans remaining solely dependent on livestock for food and financial security (African Union, 2002), BTB disease surveillance has not been maintained in rural areas. The socio-economic and public health consequences of BTB are therefore unknown.

BTB is listed as a category B disease by the Office des Epizootics, and as such requires active surveillance and reporting for effective control. However, data from many developing countries, including South Africa is incomplete or rarely available (Ayele et al. 2004). Reports from private game reserves and national parks throughout southern Africa indicate that the spread of BTB is one of the primary concerns for wildlife health and conservation (Michel et al. 2006). It may therefore be assumed that livestock and human populations in direct or environmental contact with infected wildlife are at an increased risk of BTB infection (Wray et al. 1975).

Humans contract bovine tuberculosis via inhalation or ingestion of bacilli resulting from contact with infected animal secretions or the use of contaminated meat and milk (Daborn et al. 1993). Communities with close associations to infected animals and who rely heavily on livestock for food and livelihood sustainability are at an increased risk of infection. Practical risk factors for infection include shepherding and milking of cattle and goats, sleeping in bomas for stock security, field butchery, hide production, and ritual slaughter practices. The wider human population are at risk through the widespread consumption of infected milk and associated dairy products (Karimuribo et al. 2005). Walshe et al. (1991) estimated that 90% of the total milk produced in Africa is consumed raw or soured, often within 30 minutes of milking. Analogous studies in Ghana reveal that only 17% of herdsmen boiled their milk before consumption, with no pasteurisation considered if they planned to sell it informally (Bonsu et al. 2000). Although the herd prevalence of BTB was 13.3%, only 7% of the livestock owners were aware of the possibility of tuberculosis infection in cattle. Thus, the informal use of bush meat, the trading of infected live animals and the use of contaminated meat and milk in areas with no formal disease surveillance not only promotes the transmission of BTB over a wide area, but results in a severe underestimation of disease prevalence.

No recent official data is available on zoonotic TB in South Africa. Despite the World Health Organisation identifying the possible importance of zoonotic tuberculosis in the 1950s (WHO, 1950), it was only in 1993 that a meeting was convened in order to investigate the zoonotic aspects of BTB on a global scale (WHO, 1994). Considering that two million tuberculosis cases of unknown origin are recorded in sub-Saharan Africa per annum (WHO, 2005), it can only be assumed that limited funding, resources and diagnostic capacity has prevented the initiation of the recommended enquiries in zoonotic disease.

Estimates of BTB prevalence in the human tuberculosis caseload have been published for a number of African countries, including Cameroon (4-7%) (Zinsstag et al. 2006), Egypt (6.4%) (Grange, 2001) and Nigeria (7.2%) (Zinsstag et al. 2006). However, in many areas a true picture of the zoonotic infection is not possible due to the lack of diagnostic equipment necessary for tuberculosis species identification. Regardless, when the prevalence of BTB in rural cattle is reported at levels greater than 90% in areas of Ethiopia (Yehualashet, 1995), 19.7% in Uganda (Zinsstag et al. 2006), 19% in Ghana (Zinsstag et al. 2006) and 17% in Chad (Schelling et al. 2000)

and 33% of herds were infected in Zambia (Zinsstag et al. 2006), the likelihood of transmission to humans is increased.

Predictions on the potential of zoonotic TB can be made by combining human health care data with known animal statistics. As a greater number of humans are potentially infected via the ingestion of bacilli in food, overt disease may present atypically as extra-pulmonary (EPTB) and be disseminated throughout the human body. Therefore, by monitoring trends in common BTB manifestations (e.g. cervical lymphadenitis in children) levels of undiagnosed zoonotic TB can be estimated. This can then be compared with available animal health data. For example, recent studies in Tanzania quantified 28% of human infections as attributable to BTB; 86% of which developed EPTB disease (Kazwala et al. 2005). Elsewhere, high levels of EPTB infection (South Africa, 25%; Ethiopia, 34%, Zimbabwe, 25%) (WHO, 2005) illustrates the urgency with which any links to BTB must be investigated.

Similar estimates can be made via reference to patterns of multi drug-resistant disease. BTB is resistant to a number of the leading frontline drugs used to treat TB in humans (Guerrero et al. 1997). Implications for health care include prolonged, expensive and ineffective treatments, especially in individuals co-infected with HIV (Guerrero et al. 1997). Collaborative work is therefore required to appraise the need for developments of short-course drugs for atypical human tuberculosis infections (Grange, 2001).

One further health concern is the combination of TB and HIV / AIDS infections in southern Africa, currently accounting for 86% of the global caseload (Corbett et al. 2003). As TB is the largest attributable cause of death in HIV infected individuals, an investigation into the role of BTB is certainly necessary. From a veterinary perspective, it is interesting to note two additional aspects. Firstly, that M. tuberculosis has been isolated from animals, indicating a disease feedback from humans is possible (Michel, 2002; Huitema, 1969). Secondly, that in households where humans had been diagnosed with TB in the past 12 months had a greater level of tuberculin positive animals (Zinsstag et al. 2006). This may have an impact on the broader health of livestock and wildlife, particularly in areas close to parks. For example, the number of HIV positive patients with TB around Hluhluwe-iMfolozi park in KwaZulu-Natal, South Africa increased from 6 in 1989 to 451 in 1993 (Walker et al. 2003). Considering that this area houses 100,000 cattle and a high density of goats, the potential for a fully operative disease feedback system is strong, but has not yet been quantified. This is a prime example of one region that would benefit immediately from an enhanced multi-sectoral collaborative approach to health care.

BTB RESEARCH IN SOUTH AFRICA; THE (COLLABOARTIVE) WAY FORWARD

It is easy to identify the gaps in zoonotic disease knowledge, but the means by which they should be repaired, less so. In order to understand the role of BTB in human an animal health, the first step is to quantify the prevalence of disease in wildlife, livestock and high-risk human populations. This would certainly enable the relative importance of practical risk factors and multi-directional routes to be identified and prioritized. The integration of data and expertise from relevant wildlife, veterinarian and medical specialists is therefore essential to ensure the improvement of disease control programmes and health care services.

Unfortunately, in many developing countries, the implementation of measures required for disease control is hindered by generally poor infrastructure and a lack of trained professionals. In South Africa, only 58% of the annual BCG vaccinations were administered in 2004 (WHO / UNICEF Report, 2004) and only 2306 veterinarians are registered to cover multiple-disease testing for the commercial, private and rural health sectors (Personal communication, South African Veterinary Council). It is therefore not surprising that many remote areas do not have a permanent health care service on which they can rely. Thus, under the constraints of restricted time, location and manpower resources, and increasing poverty (Woolhouse et al. 2005), it is imperative that research is conducted, and health programmes are initiated, in a collaborative manner. If applied

with sensitivity, this approach should help to identify and respond to the future public health needs of South Africa.

In order for effective disease control to move forward, it is essential that professionals involved in health care recognize the need for multi-disciplinary partnerships, and should promote these philosophies via combined training. Participants linked in any way to public health should be prepared to work in a cross-disciplinary manner and be ready to actively engage with a range of stakeholders, (Geoghegan, 2006). These should include veterinarians, medical professionals, wildlife managers, farmers, social scientists, policy makers, government officials, health economists and members of the local communities. Only once these partnerships are in place, can firm steps be taken to initiate and sustain comprehensive surveillance and monitoring programmes.

Databases that contain combined human and animal health data can facilitate the identification of potential disease hotspots. The use of GIS and spatially explicit modelling programmes can help visualize large volumes of complicated data and predict likely scenarios for specific disease outbreaks. The key factor is to develop user-friendly and easily understood media so that a range of data can be stored and accessed by an equally wide number of users. Similar systems in Cameroon have allowed BTB infection data to be compared with strain data collected elsewhere. This has shown that BTB infections resemble types found in Europe, with the prospect that current disease strains in Cameroon may have originated from this region, possibly France (Njanpop-Lafourcade et al. 2001). Potential international collaboration can then aid the implementation of suitable control and testing measures that have been effective in other areas with similar problems. This philosophy also works well within countries and regions, and helps reduce the costs of programme development if ideas can be obtained from and combined with neighbouring areas.

Although the initial costs in establishing collaborative health care may seem preventative, when the social, demographic, conservation and animal trade economics are factored into the calculations, most existing collaborative policies have proven to be very cost-effective (Zinsstag et al, 2005). However, without the sustained commitment of professional health workers, the capacity to implement disease testing, prevention and control programmes will be compromised.

In conclusion, although we have yet to conquer the rising army of preventable deaths from infectious disease, or fully understand the role of zoonosis in these figures, progress is being made. Veterinarians have been described as; “the most extensively distributed, highly educated resources in African rural areas” (Schelling et al. 2005a). It is therefore important that we use this knowledge to contribute to the advancement of ‘One Medicine’, to tackle the problem of disease in South Africa.

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WHAT IS THE ROLE OF SURFACE WATER IN THE EPIDEMIOLOGY OF BOVINE TUBERCULOSIS IN AFRICAN BUFFALO (*SYNCERUS CAFFER*)?

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ABSTRACT

African buffalo is the maintenance host for *M. bovis* in the endemically infected Kruger National Park (KNP). While the main route of transmission between buffalo is by aerosol, it is not known whether shedding of *M. bovis* in nasal and oral excretions may lead to contamination of surface water and facilitate infection in other animal species. A study to investigate the risk of water contamination with *M. bovis* was dovetailed with a BCG vaccination trial in African buffalo conducted under semi-free ranging conditions. Groups of vaccinated and nonvaccinated buffalo were kept together with known infected in-contact buffalo cows to promote natural challenge via horizontal transmission of *M. bovis.* Since no transmission could be demonstrated after a period of eleven months, all buffalo were experimentally infected with a field strain of *M. bovis* via the intra-tonsillar route. Serial water samples were collected from the drinking troughs of the buffalo once per season over a 12-month period and cultured for mycobacteria. All water samples were found negative for *M. bovis* but 15 non-tuberculous *Mycobacterium spp.* isolates were cultured. Two species were cultured from lymph node samples collected from the buffalo after slaughter. They were identified using 5'-16S rDNA PCR-sequencing which grouped them into five species, including *M. terrae, M. vaccae, M. engbaekii* and two species which have not yet been classified. The apparent lack of *M. bovis* shedding and the potential role of environmental mycobacteria as the cause of non-specific reactions in the interferon gamma assay and as BCG antagonists are discussed.

INTRODUCTION

Tuberculosis in wildlife, caused by *Mycobacterium bovis,* has emerged as an increasingly important disease of free-ranging wildlife populations (de Lisle et al. 2002, Michel 2002, Schmitt et al. 2002). The African buffalo (*Syncerus caffer)* has established itself as a maintenance host for *M. bovis* in two of South Africa's largest conservation areas, namely the Kruger National Park (KNP) and the Hluhluwe iMfolozi Park (HiP) (de Vos et al. 2001, Michel et al. 2006) (27). Transmission of *M. bovis* between herd members occurs most frequently by aerosol, whereas spillover to other species requires different modes of transmission (1) (Bengis et al. 1996). Predators and scavengers alike contract the infection commonly by ingestion of infected tissues (27) (Michel et al. 2006). Other pathways may apply only to particular animal species. The secretion of infectious pus from draining fistulae of parotid lymph glands, for example, has been suggested as a mode of transmission between greater kudu (Keet et al. 2002) and contaminated faeces have been implicated in the spread of bovine tuberculosis (BTB) from cattle to small buck (Thorburn 1940) as well as within a troop of baboons (Keet et al. 2000). Hence, environmental *M. bovis* contamination may be a side effect of events leading to spillover or it may be the cause of spillover itself. If pathogenic microorganisms can retain their viability for some time outside the animal host, environmental sources could play a significant role in their spread to a wide range of animal species from different habitats and ecological niches. To this effect it has been shown that *M. bovis* can survive for 42 days in tissues with lesions and up to four weeks in faecal material from buffalo (Tanner & Michel 1999). The

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tenacity of tubercle bacilli in effluents from sanatoria and dairies and its significance in the spread of infection to cattle were major public health concerns prior to eradication of BTB in Europe (Kraus 1942, Jensen 1954)
No information is, however, available on the role of surface water in the epidemiology of bovine tuberculosis in an endemically infected ecosystem, especially where limited water sources cause a variety of animal species to gather in high densities for most of the year.
The present study was conducted during a BCG vaccination trial in buffalo involving *M. bovis* challenge. We used the trial as an opportunity to determine whether naturally or experimentally infected buffalo were shedding detectable levels of *M. bovis* into the drinking water, and if so, to provide an estimate of the organism's tenacity. The second objective was aimed at screening the water for free-living, non tuberculous mycobacteria (NTM) which have been previously implicated in an antagonistic effect to BCG (Brandt et al. 2002, Buddle et al. 2002). Environmental mycobacteria have also been implicated in the non-specific sensitization of cattle and buffalo to the intradermal tuberculin test and the interferon gamma assay (Cooney et al. 1997, Michel 1998, Grobler et al. 2002).

MATERIALS AND METHODS

Study site

The present study was conducted in parallel with a BCG vaccination trial in buffalo, and was carried out in a 100 hectare fenced camp with natural habitat near Skukuza in the KNP. Two concrete drinking troughs (inner troughs) with a capacity of 400 liters each, were located in an enclosure situated within one corner of the camp and were the only permanent water source for the buffalo, except for small transient ponds which formed sporadically during the rainy season between the months of October and March. Fresh water was pumped daily from a tributary of the nearby Sabie River to fill both drinking troughs. A separate concrete trough (experimental trough) with a capacity of approximately 250 liters was located next to the inner troughs but on the outside of the enclosure and camp. This trough was used for collection of serial water samples as described below.

Access to the inner water troughs was restricted for several hours before each new sampling experiment to ensure that all buffalo had to consume water before sampling took place later the same day.

Animals

The study focused on naturally and experimentally infected buffalo used in the BCG vaccination trial. Shortly before the start of the trial 27 skin test and interferon gamma negative buffalo, aged about two years, were translocated from the northern part of KNP into a holding facility (boma) at Skukuza. Prior to introduction into a 100 hectares camp, 14 animals were randomly selected and vaccinated twice with BCG, 6 weeks apart.
The initial vaccination protocol anticipated natural *M. bovis* challenge from close contact with infected herd members. For this purpose a group of 27 adult buffalo cows was captured from a high prevalence herd in the south of the KNP and introduced into the same camp to join up with the 27 experimental buffalo. After a one-year period without any evidence of horizontal transmission of *M. bovis* to the nonvaccinated buffalo, both the vaccinated and control groups were challenged with a field strain of *M. bovis* via the intra-tonsillar route in January 2004 (de Klerk, unpublished data). Twenty-four of the 27 in-contact buffalo cows and all 27 experimental buffalo survived. During the trial period 16 calves were born to the in-contact cows. Three months after the last water sampling was conducted, all buffalo (n=67) were slaughtered.

Interferon gamma assay

The modified, triple comparative interferon gamma assay as described by Grobler et al. (2002), was used to determine the BTB status of the captured adult buffalo cows and to monitor the cellular

immune response of buffalo in the vaccinated and nonvaccinated groups. All experimental buffalo were tested eight times between the start of the vaccination trial in January 2003 and before slaughter of the buffalo in October 2004.

Interpretation key for IFNg assay:

Positive (M. bovis) reactor:

$$OD_{bovine} - OD_{avian} > 0.20 \text{ AND } OD_{fortuitum} - OD_{nil} < 0.15$$

Multiple reactor

$$OD_{bovine} - OD_{avian} > 0.20 \text{ AND } OD_{fortuitum} - OD_{nil} > 0.15$$

Avian reactor:

$$OD_{avian} - OD_{bovine} > OD_{bovine} + \frac{OD_{bovi}}{10}$$

Equal reactor:

$$OD_{bovine} + \frac{OD_{bovine}}{10} > OD_{avian} > OD_{bovine} \text{ or } OD_{bovine} - \frac{OD_{bovine}}{10} < OD_{avian} < OD_{bovne}$$

Water sample collection plan

One sampling experiment was conducted each in September 2003, as well as in the months of January, April, and August 2004 (TABLE 1).

At the start of each sampling experiment, the buffalo were allowed to drink from both inner troughs and subsequently chased out of the enclosure. Following mixing of the water and collection of water sample A (TABLE 1), approximately 100 liters of water was transferred manually from each inner trough into the experimental trough outside the fence, using a bucket. The water temperature in both troughs was recorded daily throughout the experiment. During each experiment, ten serial water samples, 400 ml each, were collected between 2 hours and 21 days after buffalo contact to allow an estimate of the survival time of *M. bovis*, if required. The water samples were stored at -20^{o}C until transferred to the Tuberculosis laboratory at Onderstepoort Veterinary Institute (OVI) for culture.

Tissue sample collection plan

At slaughter a standard set consisting of nine lymph node samples was collected for histopathology and culture from each buffalo. The samples included lymph nodes of the head (incl. tonsils), thorax, abdomen and carcass. Samples were also collected from any other tissues with visible lesions. All tissue samples for culture were individually packed in sterile containers and frozen at -20^{o}C until processed in the Tuberculosis laboratory at the Onderstepoort Veterinary Institute (OVI).

Bacterial isolation

Tissue samples were processed and identified according to the protocol described by Bengis et al. (1996). A modification of the standard protocol was used to isolate *M. bovis* from the water samples. Briefly, one hundred ml of water was centrifuged at 3500 rpm for 10 min and the pellet resuspended in 25 ml of sterile, double distilled water. Decontamination was effected by adding 25 ml of sodium hydroxide (4% w/v). The mixture was left for 10 minutes before centrifugation for 15 min at 3500 rpm. The pellet was neutralized by adding 5% oxalic acid for 15 minutes, followed by centrifugation as before. The pellet was mixed and inoculated onto four slants of Loewenstein-Jensen medium, two of which contained pyruvate to facilitate growth of *M. bovis*.

Identification of *Mycobacterium spp*. by polymerase chain reaction (PCR)

All acid-fast isolates were subjected to a PCR assay specific for *M. tuberculosis* complex bacteria (Cousins et al 1991). All isolates which failed to yield the expected 372 bp amplification product, were subjected to a 5'-16S rDNA PCR-sequencing assay, which is able to identify non-tuberculous *Mycobacterium spp*. (Harmsen et al 2003).

RESULTS

Animals

M. bovis infection was confirmed by culture and subsequent PCR identification of acid-fast isolates in 14 of the 24 surviving in-contact buffalo cows, of which 11 presented with macroscopic lung lesions at slaughter (de Klerk, manuscript in preparation). Five cows showed extensive lesions in the lungs and one or more lymph nodes. From the vaccinated group, seven out of eight culture positive buffalo showed visible lesions in lymph nodes of the head and thorax whereas no visible lesions were detected one buffalo. Among the nonvaccinated controls eight buffalo showed lesions predominantly in the lymph nodes of the head, while nine were culture positive. One control animal presented with a lung lesion. Two of the 16 calves which were born to the in-contact cows yielded *M. bovis* on culture, both with involvement of the thoracic lymph nodes.

Isolation and identification of *Mycobacterium spp*. by polymerase chain reaction (PCR)

M. bovis was isolated from a range of tissue samples as described under "Animals", but not from any of the water samples. However, 13 other mycobacterial isolates were recovered from water samples collected between January and August 2004, and a single NTM isolate derived from the samples collected in September 2003 (TABLE 1). Two NTM species were isolated from lymph nodes of vaccinated buffalo. These 16 *Mycobacterium spp.* isolates were acid-fast on microscopic smear examination but failed to amplify the expected 372 bp product in the PCR protocol used to identify *M. tuberculosis* complex bacteria (data not shown). Subsequent analysis using 5'-16S rDNA PCR-sequencing revealed that these organisms were all non-tuberculous members of the genus *Mycobacterium* belonging to the species *M. terrae*, *M. engbaekii*, *M. vaccae* and two previously unidentified species closely related to *M. moriokaense* and *M. kansasii* (and *M. szulgai*), respectively (TABLE 1). The NTM species isolated from the buffalo tissues were identified as *M. thermoresistibile* and an unidentified species closely related to *M. moriokaense.*

Interferon gamma assay (IFNg assay)

The IFNg test results of vaccinated and nonvaccinated buffalo on eight test occasions are summarized in TABLE 2. For the purpose of this study, the emphasis was placed on non-specific sensitization detected in 20 out of the 27 experimental buffalo. Non-specific immune reactions occurred sporadically on one or more occasions in both vaccinated and nonvaccinated buffalo and were classified, according to the method of Grobler et al. (2002), as avian, equal or multiple reactions. A general increase in the frequency of these reactions was noted starting from the test occasion in April 2004, three months after the intra-tonsillar infection of the buffalo.

DISCUSSION

Mycobacterium bovis infection in buffalo is primarily spread by the airborne route, often resulting in lesions in the upper and lower respiratory tract (de Vos et al. 2001). As demonstrated for cattle (Neill et al. 1988, 1992), infected buffalo may at times shed *M. bovis* in nasal and/or oral discharges and might therefore contaminate water points such as water holes, dams and pools formed in stagnant rivers. Buffalo herds spend considerable amounts of time in and along water and given the high BTB prevalences detected in the south of the KNP (Michel 2006), especially stagnant water sources should be considered a potential source of infection to other species. In this study, *M. bovis* contamination of water through infected buffalo was investigated. No *M. bovis* was isolated from any of the water samples collected from the drinking troughs of naturally and experimentally infected buffalo.

Although *M. tuberculosis* complex bacteria are obligate intracellular pathogens, they have been shown to maintain their virulence in watery environments including unpolluted tap water, streams

or sewage for months (Möller, 1901, Kröger & Trettin, 1951, Schmidt 1952, Genov 1965). In our study eleven naturally infected, adult buffalo cows with advanced ('open') lung lesions, which is considered a sign of infectiousness in cattle (Kleeberg, 1975, Cousins et al. 2002), presented a confirmed source of *M. bovis*. We therefore believe that our findings indicate that shedding of *M. bovis* in nasal and oral discharges is either an infrequent event in African buffalo, possibly limited to animals in very advanced or terminal stages of the disease, or does not generally lead to detectable levels of contamination in surface water. This conclusion is supported by the fact that the apparent failure of natural *M. bovis* challenge during the first phase of the vaccination trial was thought to be the consequence of a low *M. bovis* transmission rate from the infected in-contact cows to the experimental animals. On the other hand, successful transmission of *M. bovis* might not only depend on shedding of the organism but might require very close physical contact to allow successful aerosol transmission between donor and recipient. In-contact cows and experimental animals had been sourced from different herds and there is a possibility that the social interactions between them might not have been sufficient to support transmission, whereas two surviving calves of infected cows both showed signs of respiratory infection with *M. bovis*. Intra-tonsillar challenge with a field strain of *M. bovis*, resulted in macroscopic lesions in retropharyngeal lymph nodes and tonsils in 15 buffalo and culture isolation of *M. bovis* from 18 experimental buffalo (results not shown). According to Neill et al. (1988, 1992), excretion of *M. bovis* in nasal mucus is a consistent feature in all infected cattle and can continue for weeks and even months. If the same principle was true for infected African buffalo we would have expected to isolate *M. bovis* from the trough water, unless it was present in below-detectable levels. In our own experience the recovery rate of *M. bovis* from nasal swabbings taken from known infected buffalo is very low (Michel, unpublished data). Once large ecosystems become endemically infected, management options to control BTB are effectively limited to measures aimed at reducing the prevalence and transmission of the infection in the maintenance host population(s). Among the most promising interventions are culling programmes (Jolles et. al. 2005) and vaccination (Corner et al. 2002, Griffin et al. 2001). Initial vaccination trials using BCG in buffalo did not yield a significant reduction of infection, questioning the efficacy of BCG in these buffalo (de Klerk, manuscript in preparation). Demangel et al. (2005) have implied a potential adverse effect of environmental mycobacteria on vaccination efficacy of BCG, depending on the extent to which these mycobacteria share cross-reacting antigens with the vaccine. In calves, sensitization with environmental mycobacteria prior to vaccination had an antagonistic effect on BCG efficacy (Buddle et al. 2002). Our microbiological examination of water, pumped from a tributary of the Sabie River into the water trough for the study buffalo, yielded five species of NTM including two previously unidentified species (TABLE 1). *M. vaccae* and *M. terrae* are reportedly among the most frequently isolated organisms from fresh water (Viallier & Viallier 1973), along with *M. engbaekii* and a number of unclassified mycobacteria (Marranzano 1978). Their natural habitat, however, is more likely to be wet soil (Collins & Grange 1984), which may suggest that the NTM did not all originate from the river water but could have been introduced via the soiled muzzles or feet of the buffalo while drinking. Favourable water temperatures throughout the experiments (TABLE 1) and the presence of sufficient nutrients in river water are known to facilitate replication of mycobacteria (Collins et al. 1984). It may be speculated that the isolation of certain Mycobacterium species, e.g. *M. engbaekii* preferrably during the latter part of the experiments might have depended on these as enabling conditions.

In contrast to members of the *M. tuberculosis* complex, NTM are rarely associated with invasive disease, but they may temporarily colonise the host and cause transient infection accompanied by non-specific stimulation of the host's immune system (Woods & Washington, 1987). In our study we isolated an unidentified Mycobacterium species closely related to *M. moriokaense* from both the trough water and lymph node tissue from one of the buffaloes.. Although it remains to be proven, it is tempting to speculate that one or more of the NTM species isolated from the trough water caused the non-specific reactions detected in the experimental buffalo using the IFNg assay (TABLE 2). In previous reports, various NTM species, including *M. vaccae* (Shimizu et al. 1977) and *M. kansasii* (Bercovier & Vincent 2001), have been implicated in false positive test results in the intradermal

tuberculin test and the IFNg assay in different countries (Corner & Pearson, 1978, Cooney et al. 1997, Grobler et al. 2002).

In summary, the findings of this study suggest that buffalo do not significantly contribute to the spread of *M. bovis* through contamination of water. We also demonstrated that buffalo were exposed to different NTM in river water without producing any signs of disease. Further investigation is required to elucidate the role of environmental mycobacteria especially in the immune response of buffalo to virulent *M. bovis* as well as its effect on BCG vaccination.

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THE IMPACT OF 2 DIPPING SYSTEMS ON ENDEMIC STABILITY TO BOVINE BABESIOSIS AND ANAPLASMOSIS IN CATTLE AT 4 COMMUNALLY GRAZED AREAS IN LIMPOPO PROVINCE, SOUTH AFRICA

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ABSTRACT

A 12-month study was conducted at 4 communal grazing areas in the Bushbuckridge region, Limpopo Province, South Africa. The main objective was to investigate the impact of reduced acaricide application on endemic stability to bovine babesiosis (*Babesia bigemina* and *Babesia bovis*) and anaplasmosis (*Anaplasma marginale*) in the local cattle population. To this end 60 cattle at each communal grazing area were bled at the beginning and the conclusion of the experimental period and their sera were assayed for *B. bovis*, *B. bigemina* and *Anaplasma* antibodies. Cattle in the intensively dipped group were dipped 26 times and maintained on a 14-day dipping interval throughout the study, whereas cattle in the strategically dipped group were dipped only 13 times. Three cattle, from which adult ticks were collected, were selected from each village, while immature ticks were collected by drag-sampling the surrounding vegetation. During the dipping process, a questionnaire aimed at assessing the prevalence of clinical cases of tick-borne disease, abscesses and mortalities was completed by an Animal Health Technician at each diptank.

An increase in seroprevalence to *B. bovis* and *B. bigemina* and a decrease in seroprevalence to *Anaplasma* was detected in the strategically dipped group whilst in the intensively dipped group the converse was true. *Amblyomma hebraeum* was the most numerous tick species on the cattle, and *Rhipicephalus (Boophilus) microplus* was more plentiful than *Rhipicephalus (Boophilus) decoloratus*. Drag samples yielded more immature stages of *A. hebraeum* than of *Rhipicephalus (Boophilus*) spp. The incidence of clinical cases of tick-borne disease and of abscesses increased in the strategically dipped group at the start of the survey.

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INTRODUCTION

Tick infestation and the diseases transmitted by ticks are a major problem for farmers in the tropical and subtropical regions of the world and are widespread in Africa[12,26]. Ticks and tick-borne diseases (TBD) cause considerable economic losses to owners in those areas of southern Africa in which cattle are communally grazed, and the occurrence of tick worry, abscesses and even mortality is often high[31].

Three economically important TBD occur in the region, namely bovine babesiosis, caused by *Babesia bovis* and *Babesia bigemina*, bovine anaplasmosis caused by *Anaplasma marginale*, and heartwater or cowdriosis, caused by *Erhlichia* (*Cowdria*) *ruminatium*. *Babesia bovis* is transmitted by *Rhipicephalus* (*Boophilus*) *microplus*, the only known vector in southern Africa[38], *B. bigemina* by *Rhipicephalus* (*Boophilus*) *decoloratus* and *R.* (*B.*) *microplus*[30] and to a much lesser extent by *Rhipicephalus evertsi evertsi*[7] and *A. marginale* chiefly by *R. (B.) decoloratus* and *R. (B.)*

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microplus, and to a lesser extent by *Hyalomma marginatum rufipes, R. evertsi evertsi* and *Rhipicephalus simus*. Mechanical transmission by biting flies also occurs. In South Africa *E.* (*Cowdria*) *ruminantium* is transmitted by *Amblyomma hebraeum*.

Livestock production in southern Africa is heavily dependent on improved animal health and this entails good tick and TBD control. Many commercial and some rural subsistence farmers use regular short-interval dipping to keep their cattle virtually tick-free. Intensive dipping policies were first instituted during the East Coast fever (*Theileria parva*) era and this has led to endemic instability to many of the TBD[31]. More recently, however, there has been a shift towards strategic and threshold tick control with acaricides applied less frequently during periods of low tick abundance and more frequently during the critical times of the year to avoid the damaging effects of adult ticks[15,29].

In the Bushbuckridge region, farmers were previously dependent on the Department of Veterinary Services in Limpopo Province, South Africa for the free of charge provision of acaricide, maintenance of diptanks and for supplying diptank personnel and other labour. In extensive beef production systems, especially on commercial ranches in southern Africa, the gathering of cattle for dipping is labour-intensive and costly[31]. Furthermore, farmers may suffer production losses as a result of stress, abortions, drowning and physical injuries and may also lose a day's animal traction and labour on dipping days. Many commercial beef farmers would like to move away from intensive dipping, but are not convinced that alternative control strategies are cost-effective and do not carry a high risk of outbreaks of TBD[32].

The afore-mentioned are some of the important reasons for re-appraising present-day tick control strategies in Africa. The alternative approaches emphasize the maintenance of endemic stability (seventy five percent of animals in a herd are seropositive), the use of vaccines against TBD, and the introduction of tick resistant cattle. Where vector density is high, infection with TBD is common and usually occurs early in the host's life accompanied by reduced morbidity and mortality[31,34]. Stability to bovine babesiosis, anaplasmosis and heartwater is common in endemic areas in Africa[10,30]. Infestation with *R.* (*B.*) *decoloratus* usually indicates endemic stability to *B. bigemina*[11,30], thus reducing the risk of losses due to the parasite. In a study conducted in South Africa, cattle that were treated erratically with an acaricide or on which there was a reduction in the intensity of acaricide application, passed from endemic instability and low prevalence of seropositivity to endemic stability and high prevalence of seropositivity without outbreaks of clinical disease[42]. On the other hand intensive dipping interferes with the development of endemic stability to TBD[8].

There are now many proponents of the view that it is advisable to aim for endemic stability to TBD in communally grazed areas in Africa because it is the more sustainable option[30,31].

Frozen, live, blood-based vaccines against bovine babesiosis and anaplasmosis are presently available[4,11], and a single vaccination should provide long-lasting protection. Research is now focused on the use of indigenous African cattle (*Bos indicus*), which are more resistant to ticks than European breeds[36,39]. This characteristic is attributed to both innate resistance[5,14,39] and the genetic ability to acquire resistance[4]. Tick resistant cattle are able to attain a state of endemic stability to most TBD without tick control measures being applied[30]. Endemic stability is maintained by continuous exposure of the cattle to infected ticks; young calves become infected and are able to take advantage of an age-related resistance or colostral immunity, which minimizes the effects of the disease[31]. A recombinant DNA vaccine against the one-host tick *R.* (*B.*) *microplus* has also been developed[9,45] and registered for commercial use in Australia[45]. It targets the gut cells, destroying the tick's digestive tract and resulting in a 90% reduction in its weight and egg production capacity[17,45]. Vaccination with recombinant gut antigens of *R. (B.) microplus* has controlled tick infestations in South America[17].

It is likely that in the future communally-grazed indigenous cattle will probably require minimal tick control, ranging from none during the dry season to strategic and threshold control during the wet season[27]. Strategic control would be applied only at critical times of the year to minimize the seasonal damage caused by adult ticks. Most economically important tick species display a clear seasonal pattern of adult activity, which makes the idea of strategic control feasible[2,20,21]. Threshold tick control, which is applied only when the number of ticks per individual host exceeds a predetermined economic threshold is now also widely used[8]. It has been found that seasonal peaks in the numbers of immature *A. hebraeum* and *R. appendiculatus* occurred in spring and autumn, while all stages of *R. (B.) decoloratus* peaked in spring and late summer[2]. It was also found that immature *R. appendiculatus* peaked in spring and autumn and the adults of *Hyalomma* spp. in summer[40]. These studies indicate that dipping during peak tick activity should control those tick species prevalent in large parts of this region[13].

The primary objective of the present study was to investigate the impact of reduced acaricide application on endemic stability to bovine babesiosis and anaplasmosis in the local cattle population in the Bushbuckridge region of the Limpopo Province and to implement alternative measures to maintain endemic stability to TBD without resulting in a significant increase in their occurrence and of tick damage.

MATERIALS AND METHODS

The study was conducted at four communal grazing areas (CGA) at Bushbuckridge, namely Oakley (31°15′S, 24°58′E), Cunningmore (31°16′S, 24°56′E), Mkhuhlu (31°16′S, 25°00′E) and Ronaldsy (31°18′S, 24°55′E). Fences separated the farms but all four CGA were located in a single ward and had similar vegetation and climatological and ecological conditions. This is a summer rainfall area with high tick challenge during the summer months (November to February) and the grazing consists of natural sourveld[1]. Two hundred and forty, predominantly Nguni cattle, aged between 6 months to fully grown adults were selected from the four CGA (60 animals per CGA). One hundred and sixty of these were older than 2 years and 80 were calves less than 1 year old. The adult cattle and calves were selected for the experiment by a random sampling technique[41]. The four diptanks in the study region were the primary sampling units and the individual cattle/calves were the secondary sampling units[41]. The prevalence of bovine babesiosis and anaplasmosis in the study region was unknown, hence an estimation of a 25% prevalence with a 95% confidence level was made. The sample size was determined by assuming that the estimated prevalence was within 5% of the true level[41].

At Cunningmore and Mkhuhlu 120 cattle were dipped intensively, i.e. at 14-day intervals, while a similar number at Ronaldsy and Oakley were dipped strategically. All adult cattle and calves in the study area had been dipped at 14-day intervals prior to the start of the experiment. The 2 groups of the sample population were run in separate but similar grazing camps from April 2002 to March 2003. Blood samples were collected from 240 animals during April 2002 and again from 240 animals (not necessarily the same animals) during March 2003 and the sera were assayed using the IFAT for *B. bovis* and *B. bigemina*[23] and the CI- ELISA for *A. marginale*[44].

Adult ticks were collected at monthly intervals prior to dipping from three animals (a calf and two adults) at each village. The animals were restrained in a crush-pen and adult ticks were collected from one half of the animal for identification and counting. The numbers recovered were multiplied by 2 to give the total number of ticks on each animal. Immature ticks were collected by drag sampling the vegetation of the four CGAs, and three drag-samples per month were done per village[35].

The intensively treated group was dipped 26 times during the study period and the strategically treated group 13 times during the same period. The strategically treated group was allowed to acquire moderate to heavy tick burdens between acaricide treatments, especially during peak adult tick activity. A questionnaire aimed at estimating the damage caused by adult ticks, including abscesses, clinical disease and mortality was completed by the Animal Health Technician stationed at each diptank during dipping.

Statistical analyses of the results were performed at the Department of Statistics at the University of Pretoria using the Chi-square test to test for association between 2 variables (SAS v 8.2 programme). If the test gave a p-value of less than 0.05 the association between the two variables was significant at the 95% confidence level. A low p-value also indicated that the association was not due to random error or to chance. The tests were performed using 2-way frequency tables and the serological data from the cattle and calves were compared for 2002 and 2003 taking the age of the animals and the dipping intervals into account.

RESULTS

Serology

The results of the serological tests for *B. bovis, B. bigemina* and *Anaplasma* for the strategically and intensively treated groups of cattle are illustrated in Fig 1.

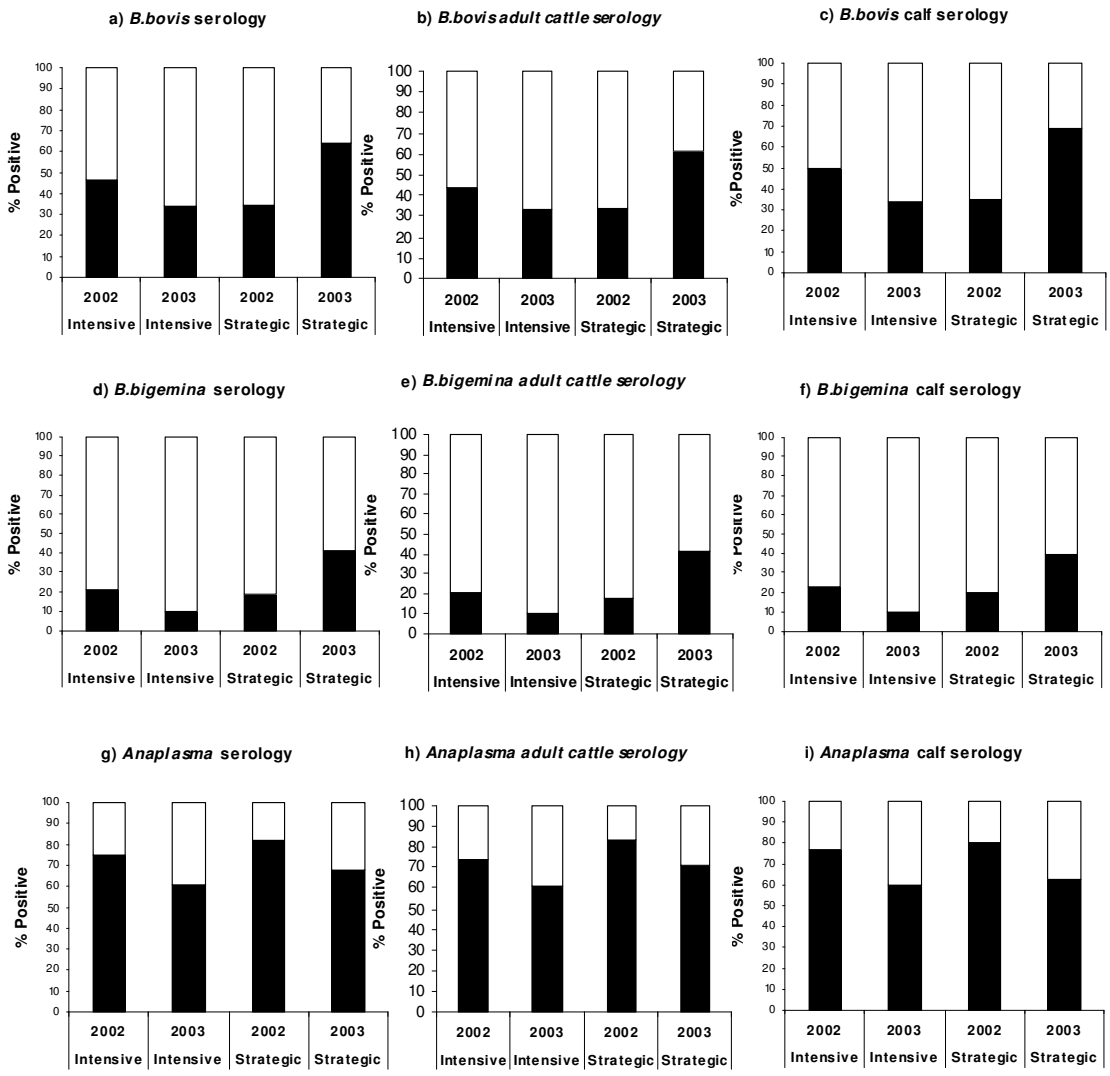


Fig 1: Comparison of the seroprevalence of *B. bovis*, *B. bigemina* and *Anaplasma* of adult cattle and calves dipped intensively and strategically between April 2002 and March 2003

a) Total *B. bovis* serology d) Total *B. bigemina* serology g) Total *Anaplasma* serology
b) B. bovis adult serology e) *B. bigemina* adult serology h) *Anaplasma* adult serology
c) *B. bovis* calf serology f) *B. bigemina* calf serology i) *Anaplasma* calf serology

The percentage of sera positive for *B. bovis* in the strategically treated group increased significantly in 2003 compared to 2002 in both adult cattle and calves ($p<0.05$). Seroprevalence for *B. bigemina* also increased significantly in the adult cattle ($p<0.05$) in 2003, but the increase in the calves was not significant. Seroprevalence for both *B. bovis* and *B. bigemina* did not change significantly from 2002 to 2003 ($p>0.05$) in the intensively treated group. There was a significant decrease in the seroprevalence of *anaplasma* in 2003 when compared to 2002 ($p<0.05$)

Ticks on cattle and the vegetation

The total counts of adult and immature ticks from cattle, calves and the vegetation for the intensive and strategically treated groups have been pooled separately and are presented in Table 1 and Figs 2, 3 and 4. *A. hebraeum* was the most common tick species collected with *R. (B.) decoloratus, R. (B.) microplus, R. appendiculatus* and *Hyalomma marginatum rufipes* also present. Adult ticks peaked during spring and summer whilst immature ticks peaked during autumn and spring (p-value of less than 0.05).

Table 1: Total number of adult ticks (accumulated data) collected from adult cattle (a+b) and calves(c+d) (2002/2003) and immature ticks collected from the vegetation

a) Intensive group (adult cattle)

R. (B.) microplus/ R. (B.) decoloratus			*A. hebraeum*			*R. appendiculatus*			*H. marginatum*		
mal	fem	imm	mal	fem	imm	mal	fem	imm	mal	fem	imm
45	46	851	45	50	1243	43	51	639	45	46	678

b) Strategic group (adult cattle)

R. (B.) microplus/ R. (B.) decoloratus			*A. hebraeum*			*R. appendiculatus*			*H. marginatum*		
mal	fem	imm	mal	fem	imm	mal	fem	imm	mal	fem	imm
43	67	953	115	120	1630	65	66	893	43	62	899

c) Intensive group (calves)

R. (B.) microplus/ R. (B.) decoloratus			*A. hebraeum*			*R. appendiculatus*			*H. marginatum*		
mal	fem		mal	fem		mal	fem		mal	fem	
43	39		46	45		57	55		48	72	

d) Strategic group (calves)

R. (B.) microplus/ R. (B.) decoloratus			*A. hebraeum*			*R. appendiculatus*			*H. marginatum*		
mal	fem		mal	fem		mal	fem		mal	fem	
51	68		102	123		63	64		40	51	

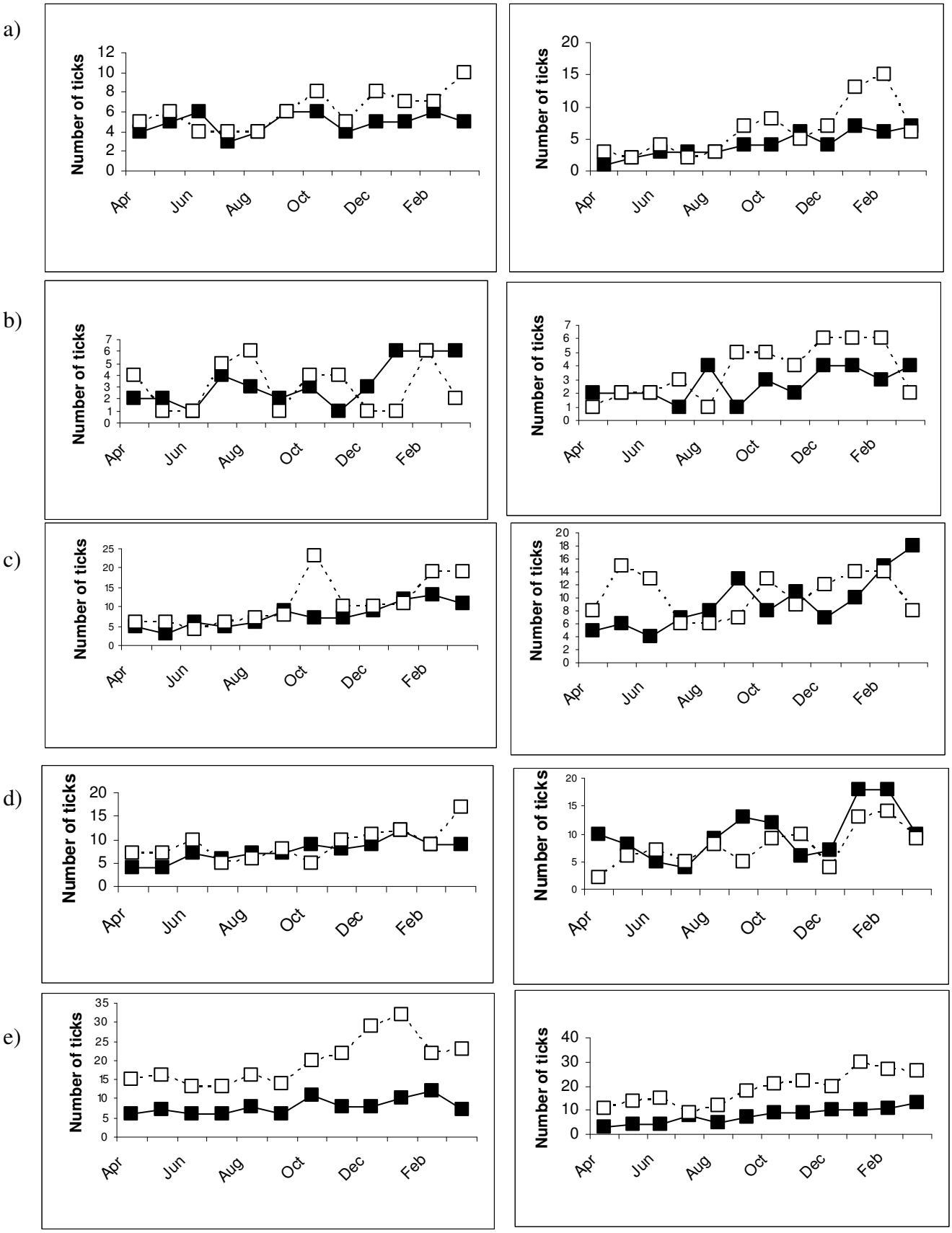
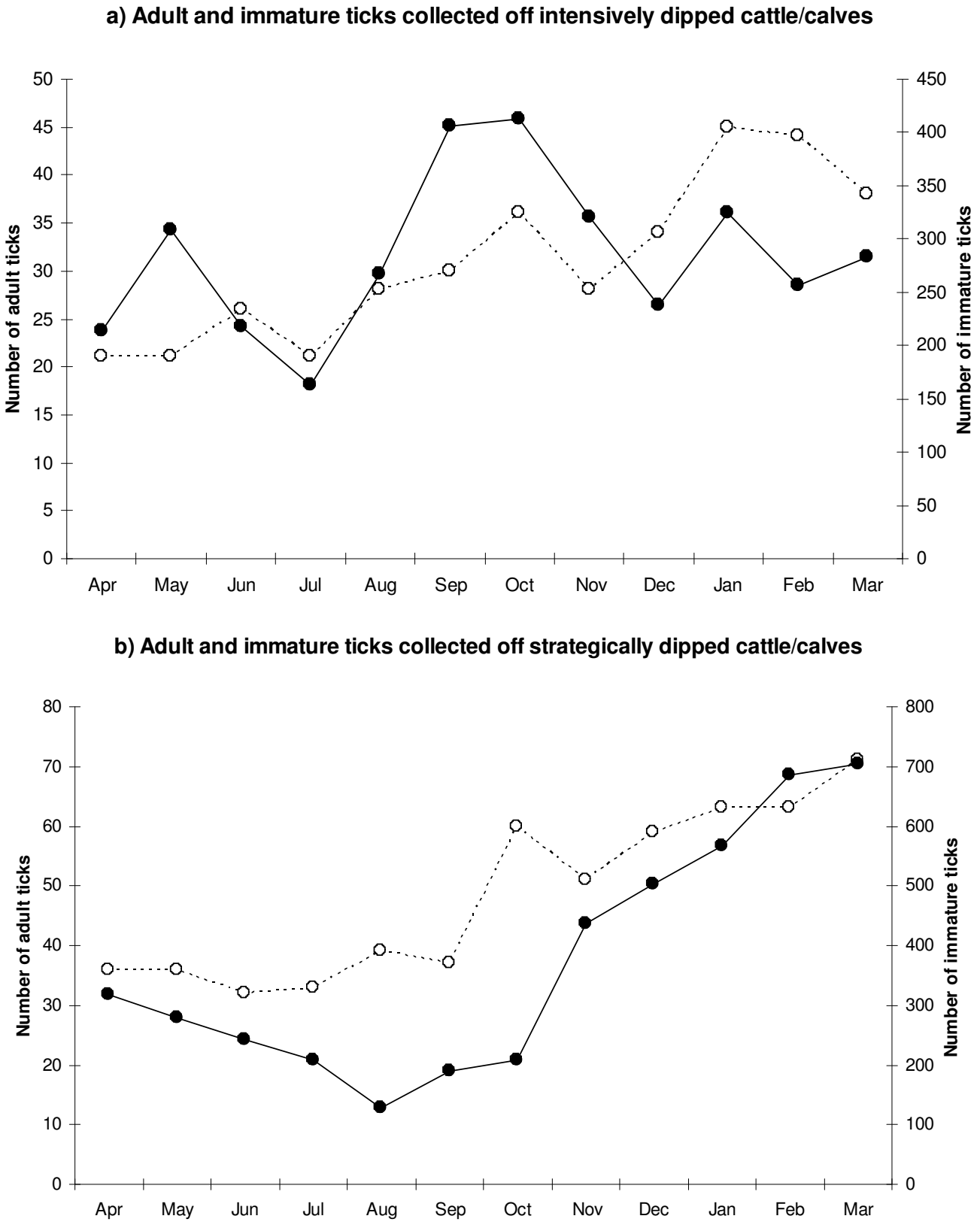


Fig 2: Adult ticks collected from intensively (□■□) and strategically (--□--) dipped adult cattle and calves from April 2002 to March 2003
a) *R. (B.) microplus* b) *R. (B.) decoloratus* c) *R. appendiculatus* d) *H. marginatum*
e) *A. hebraeum*



Fig

Fig 3: Comparison of the total adult (--○--) and immature (□●□) ticks collected off intensively (a) and strategically (b) dipped cattle and calves from April 2002 to March 2003

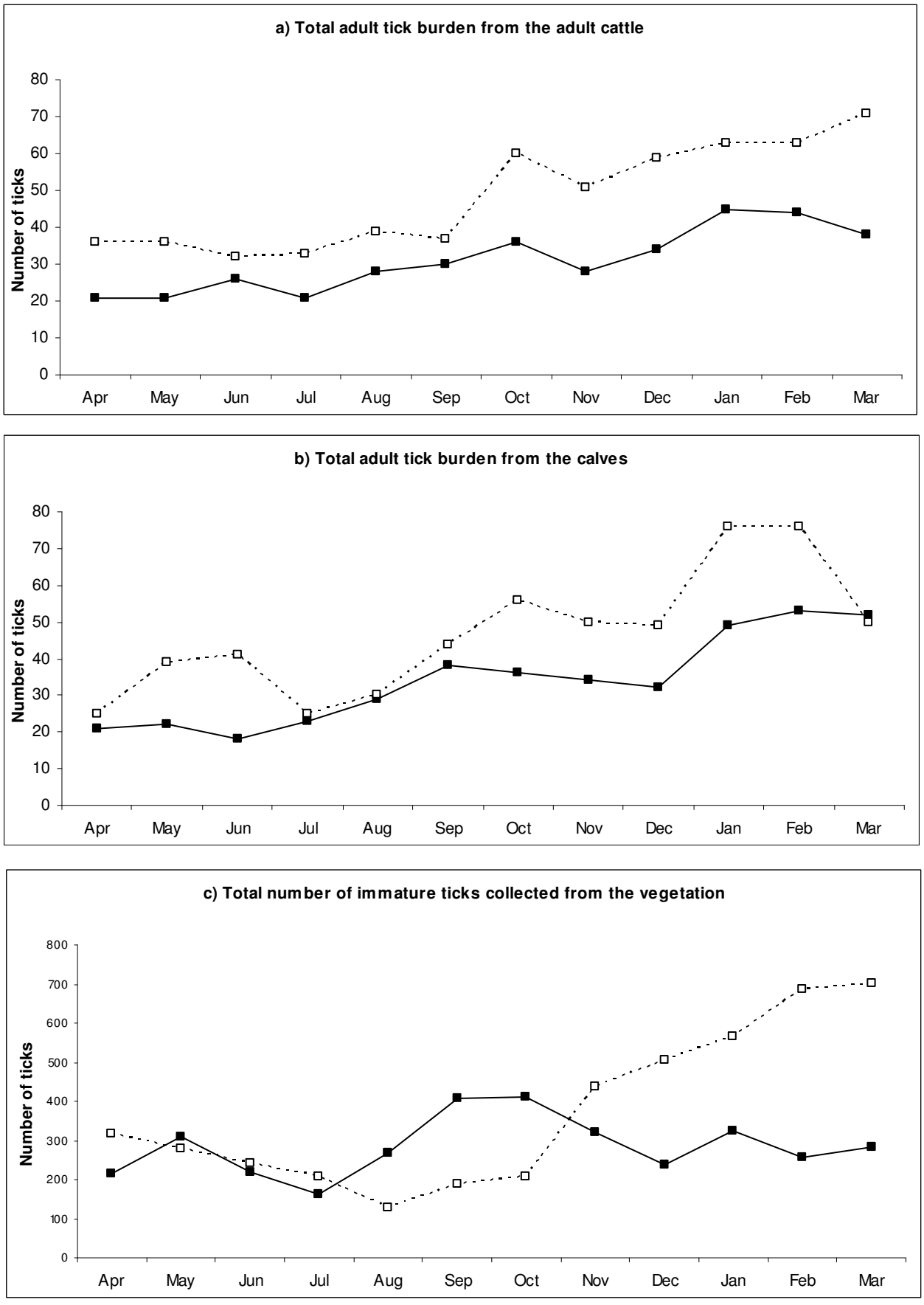


Fig 4: Total adult and immature ticks collected off: adult cattle (a) and calves (b) which had been intensively (□■□) and strategically (--□--) dipped from April 2002 to March 2003. The total number of immature ticks collected off the vegetation (c) is also included

The prevalence of clinical disease, mortality and abscesses

Three clinical cases of babesiosis (*B. bigemina*) and 9 cases of anaplasmosis were recorded in the strategically dipped group whilst only 1 case of anaplasmosis was reported in the intensively dipped group during the survey. A further 3 mortalities due to anaplasmosis were recorded in the strategically dipped group and one in the intensively dipped group. Seventeen abscesses were recorded in the strategically treated group and only two in the intensively treated group (Table 2). The TBDs in affected animals were diagnosed by a veterinarian, who used a combination of clinical signs and the microscopic examination of blood smears.

Table 2: Summary of the prevalence of clinical disease, mortality and abscessation in the cattle/calves at Bushbuckridge (number of clinical cases in each case is also indicated)

Dipping regime	Clinical disease	Mortality	Abscessation
Intensive group	Anaplasmosis (1)	Anaplasmosis (1)	Abscesses (2)
Strategic group	Anaplasmosis (9) Babesiosis (3)	Anaplasmosis (3)	Abscesses (17)

DISCUSSION

It is generally accepted that endemic stability to TBD exists when the number of seropositive animals in a herd reaches seventy five percent by nine months[25,30]. Mahoney and Ross's model was developed using serological results from calves up to 9 months of age. Older cattle were included in the present study to give a more realistic idea of the risk of disease outbreaks in the area[33]. The statistically significant increase in seroprevalence to *B. bovis* in both adult cattle and calves ($p<0.05$) in 2003 at Oakley and Ronaldsy, where the strategically treated groups grazed, is similar to findings in other surveys in southern Africa in which *B. bovis* seroprevalence was high and where *R.* (*B.*) *microplus* was common and little tick control was practiced[30,33,37]. The main cause of the increase in seroprevalence was probably due to the reduced frequency of dipping which resulted in a greater number of ticks on the cattle, thus increasing the rate of transmission of *Babesia*[33,37]. The seroprevalence levels of *B. bovis* in both calves and adult cattle in the intensively treated group (Cunningmore and Mkhuhlu) declined during the study probably because this group remained on the same intensive dipping regimen that had been practiced previously[28]. In addition the decline was probably due to a greater compliance by farmers in bringing their cattle for dipping when compared to previous years.

The significant increase in seroprevalence to *B. bigemina* in adult cattle in 2003 is consistent with findings on farms with medium tick control in Zimbabwe[30]. The increase in seroprevalence to *B. bigemina* in the strategically treated group is also probably due to an increase in tick burdens on the cattle (Fig. 3 and 4) especially *R.* (*B.*) *decoloratus* and *R.* (*B.*) *microplus.* The overall seroprevalence to *B. bovis* was higher than that to *B. bigemina* in the strategically treated group than in the intensively treated group, whereas one would expect the converse to be true[10,11]. It is common knowledge that the sensitivity of IFAT to *B. bovis*, *B. bigemina* and *A. marginale* regresses with time after exposure but it flattens out after 98 days[19,24]. It has also been shown that a serological cross-reaction between *B. bovis* and *B. bigemina* exists[3,18,19]. At OVI, positive IFAT control slides that are very specific for *B. bovis* and *B. bigemina* were used, therefore the positives were definite (Mathee, personal communication). However, several studies in Africa[30,33,34] have reported higher prevalence rates to *B. bovis* than to *B. bigemina* in communal herds where both *B. bovis* and *B. bigemina* co-exist. This could be due at least in part to the displacement of *R.* (*B.*) *decoloratus* by *R.* (*B.*) *microplus*[43] and the fact that *R.* (*B.*) *microplus* feeds more efficiently on cattle than *R.* (*B.*) *decoloratus*. If one uses the criteria suggested by Mahoney and Ross, then endemic stability to *B. bovis* and *B. bigemina* was not present in this cattle population[25].

Unfortunately an exact comparison between the 2002 and 2003 serological results for *Anaplasma* could not be made because the laboratory at the Onderstepoort Veterinary Institute, where the tests were done, used different antigenic kits for the 2002 and 2003 tests. Despite an increase in the number of *R.* (*Boophilus*) spp. ticks on the strategically treated group there was a sharp decline in the seroprevalence to *Anaplasma*. *Anaplasma marginale* is also mechanically transmitted by biting flies whose behaviour is greatly influenced by climatic conditions. However, the degree of endemic stability to anaplasmosis does not necessarily correlate with dipping frequency and it has also been stated that dipping frequency does not reduce the seropositivity to *Anaplasma*[30]. The absence of outbreaks of clinical disease prior to the study could possibly be ascribed to the presence of non-pathogenic strains of the organism in the study region. Cattle in the villages may also have been resistant to TBD after years of exposure to these diseases.

During the study adult tick numbers peaked in spring and summer (Fig 4), and statistically significant peaks in immature tick numbers occurred in autumn and in spring ($p < 0.05$). There was, however, no statistically significant correlation between the adult tick burdens of the adult cattle and those of the calves. The relationship between the seasonal counts of immature ticks and the two dipping regimens was, however, statistically significant ($p<0.05$). The most prevalent tick species collected from the cattle was *A. hebraeum*, a finding similar to that of other studies done on cattle in the Eastern Cape Province[16,21]. After *R.* (*B.*) *decoloratus*, *A. hebraeum* was the most numerous tick species on kudus in the Kruger National Park, which is adjacent to the study sites[22]. *Rhipicephalus* (*B.*) *decoloratus, R.* (*B.*) *microplus*, *R. appendiculatus* and *Hyalomma marginatum rufipes,* were also collected in significant numbers. The results are also similar to those of another recent survey in this region[5]. The presence of adult *R.* (*B.*) *microplus* recorded in the present survey might also explain the increase in seroprevalence to *B. bovis*, a finding similar to the one done in the Limpopo Province[43]. The number of *R.* (*Boophilus*) spp. collected from the cattle peaked during spring and autumn, a common finding in other regions of South Africa[2,6,35].

Eleven cases of clinical TBD occurred in the strategically treated group during the first quarter of the study period. This group of cattle had been maintained on a 14-day dipping interval prior to the study. A single case of anaplasmosis was recorded in the intensively dipped group of cattle during the corresponding period. The low incidence of clinical anaplasmosis could be due to a greater degree of endemic stability as suggested by the 75 % positive seroprevalence. All twelve clinical cases were treated and eight recovered.

It is concluded that it is unnecessary to dip intensively at fortnightly intervals in this region especially when one considers the relatively low tick burdens on the cattle and on the vegetation. The increase in seroprevalence to *B. bovis* and *B. bigemina* in the strategically treated group of cattle implies that if a reduced dipping frequency could be maintained for long enough an endemically stable disease situation should result. Outbreaks of clinical cases of disease could be treated and vaccination could be used to supplement the natural tick challenge if it is not sufficient to maintain endemic stability. The increased seroprevalence to both *B. bovis* and *B. bigemina* in calves suggests that calf vaccination is unnecessary and that tick control should therefore be aimed mainly at preventing excessive tick worry.

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APPLICATION OF VETERINARY EXTENSION METHODS TO FACILITATE STRATIFIED RANDOM SAMPLING OF CATTLE AND TICKS DURING INVESTIGATION INTO THE EPIDEMIOLOGY OF HEARTWATER (*EHRLICHIA RUMINANTIUM*)

H.C. Steyn[1], C.M.E. McCrindle[2] & M. van Kleef[1]

ABSTRACT

Veterinary epidemiology is concerned with the level and spread of disease in animal populations. Traditional methods of quarantine and slaughter or vaccination are no longer sufficient for disease control, particularly in Africa, for a multitude of reasons. Since the advent of biotechnology and genomics, molecular epidemiology has evolved, using the methodology of traditional epidemiology, coupled with the new molecular techniques to better define animal diseases and control them. Only minimum epidemiological information is available from areas particularly badly affected by the presence of heartwater in both the commercial and communal farming sectors in South Africa. Therefore an in-depth epidemiological study on *Ehrlichia ruminantium* in livestock is being undertaken in heartwater endemic regions of South Africa. Three stage random cluster sampling methods are being used for selecting geographical areas, animals and ticks. The constraints to rigorous application of random sampling under field conditions were described and discussed.

A complete epidemiological picture can only be obtained if rural communities and veterinary staff are involved in the survey. Field studies based on participatory methods are being used. This paper describes the development and field use of a skills training program for para-veterinary field staff and livestock farmers in the collection and dispatch of bovine blood and ticks (*Amblyomma spp)* sampled.

INTRODUCTION

The rickettsial agent *Ehrlichia ruminantium* is the causative agent of the disease known as heartwater or cowdriosis and is transmitted to livestock and wild ungulates by the *Amblyomma* tick species (Cowdry, 1925a and b). *Ehrlichia ruminantium* is an obligate intracellular parasite that is known to infect endothelial cells (Cowdry, 1925a). Traditionally cattle, sheep and goats have been the central focus of livestock owners in South Africa and contribute greatly to rural livelihoods. One of the major constraints to the survival of these three domestic animals is heartwater. It is on the OIE's list of notifiable diseases because of its economic impact on rural livelihoods in Africa on multiple ruminant hosts (http://www.oie.int/eng/maladies/en_classification.htm).

New technology for diagnosis of heartwater allows for better epidemiological surveillance and monitoring. However this can only be achieved if the rural communities that own the majority of animals in South Africa can be taught to recognise the disease identify the *Amblyomma hebraeum* tick, and to report heartwater cases with the assistance of the state employed animal health technicians (AHT). Once the sampling is done, the test (pCS20 real-time PCR) that was used for diagnosis and monitoring has to be optimised and this can be done using field samples. These two together are important for studying the overall epidemiology of heartwater in South Africa. Through planning of extension strategies and skills training of AHT, sufficient data can be obtained from field studies to elucidate the ecology, transmission and prevalence of heartwater in both livestock and the tick vectors using new PCR technologies.

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Bembridge (1991) has described the methods used for agricultural extension and these have been modified for use in veterinary science and animal health (McCrindle, 2002). In terms of extension and skills training it is important to delineate and describe the target audience (Bembridge, 1991; McCrindle 2002, 2004). In this study, the target audience was the AHT and the rural livestock farmers they serve. The characteristics of the AHT as a target audience are that they are employed by the state veterinary services and usually speak the local languages and understand local cultures and norms of the farmers they serve. The gender is both male and female and their age is adult (under 65). The level of education is tertiary; this course is presented by the University of the North West and UNISA (South African Veterinary Council, 2005). The rural farmers, as target audience, cover a wide spectrum, from highly educated commercial livestock farmers to communal and subsistence farmers with very little education and a low socio-economic base.

This study incorporates training and capacitation of AHT in correct techniques for collection of blood and ticks from livestock and correct methods of labelling, handling and dispatch to Onderstepoort Veterinary Institute. Three stage cluster sampling techniques for areas, animals and ticks used were used in accordance with those recommended by Cameron (1999) and Thrusfield (1995). The aim of this study was to describe the methods and constraints in the random sampling of susceptible livestock in rural areas of the state vet district of Mpulmalanga, as well as the skills training and capacitation of AHT in correct sample collection and handling.

MATERIALS AND METHODS

Study area

Heartwater is endemic in rural areas in the following provinces: Eastern Cape, Kwa-Zulu Natal (KZN), Mpumalanga, Limpopo (Coetzer, *et al.*, 2004). This study was done in Mpumalanga and the results will be modified to fit the training and sampling profiles in the other provinces.

Skills training program

The skills training of the AHT and farmers were based on situational appraisal of their training needs. An extension programme and extension materials were individually tailored to farmers or AHT. In the case of farmers, this will include knowledge of the symptoms and importance of heartwater in their stock as well as recognition of the vector, *A. hebraeum.* In the case of the AHT, skills training was presented on the humane handling of livestock, recognition and collection of ticks, correct bleeding techniques and the correct handling, labelling and dispatch of samples (Cameron, 1999). Participatory methods were used (Bembridge 1991). As previous success has been achieved with group training of AHT using photographs compiled in flip-files or computer generated graphics programmes (Microsoft Powerpoint®), this method was chosen for producing extension material (McCrindle, 2004).

Sampling

The epidemiological sampling method chosen was random three stage cluster sampling. Because both the human and animal populations in rural areas in South Africa are mobile and it is therefore very difficult to reliably estimate true population size, stratified sampling with proportional allocation would have been almost impossible (Thrusfield, 1995). In Mpumalanga, communal cattle are clustered around dip-tanks, where they are regularly gathered for surveillance by the state veterinary services as this is a high-risk area for foot and mouth disease (Coetzer, *et al.,* 2004). The three strata chosen were therefore dip-tanks, animals and ticks. Commercial farmers in this area, also each have a dip-tank, so these can also be used as sampling units.

Blood and tick collection

Sheep and goats were restrained manually using accepted humane handling techniques and 10 ml of blood was collected from the left jugular vein using 18 gauge Vacutainer needles and 10 ml

capacity EDTA tubes. Cattle were restrained in a crush-pen using accepted humane handling techniques and blood was drawn from the ventral coccygeal vein using 18 gauge Vacutainer needles and 10 ml capacity EDTA tubes. The median coccygeal artery and vein lie on the ventral side of the coccygeal vertebrae. Samples are taken from the artery or vein at the level of the third or fourth coccygeal vertebra. The needle should be inserted ventral to the vertebral body (not ventral to inter vertebral joint) in case of slippage in this way no nerve damage will occur. All tubes must be correctly labelled with the number of the animal as well as the location. A random sample of five adult (or nymphs if no adults available) *A. hebraeum* ticks were collected from each animal that is bled. Ticks from the same animal were collected and stored in a correctly labelled screw-topped plastic container with holes in the lid and kept in a cool bag for transport.

Transporting of samples

Samples were sent or transported according to the Dangerous Goods Packaging regulations as per UN class 6.2, when transported by road (SANS 10229-1:2005 edition 1).

The specimens must be declared as animal material transported for research and diagnosis. Blood samples should be identified as: blood samples from healthy animals or sick animals. The parcel should be labelled and each blood sample should be wrapped separately in order to prevent contact between them. A triple layer packaging system is needed (Absorbent, leak-proof bag, cool bag or freezer) to meet the relevant Packing Instruction. The documentation accompanying the parcel must include delivery address, sender's details, emergency contact details (name and telephone number) and clearly marked as Diagnostic specimens (Laboratory Specialist Services (Pty) Ltd).

RESULTS

Skills training program

A participatory situational appraisal was done during sampling of livestock, of the level of knowledge and skills of AHT in Nelspruit district of Mpumalanga. It was observed that AHT did not have sufficient skills in handing livestock humanely, bleeding livestock and correct handling of samples. For instance animals were not always correctly restrained. Sometimes, after blood samples were collected, they were put in a plastic bag and left in the sun. Ticks were also incorrectly collected and there were some problems with labeling. The deficiencies were broken down into achievable outcomes and a template developed, in line with that described by McCrindle (2004) for dairy farming extension. Photographs were taken on station to show exact steps in the process and these were then used to develop extension material. The extension material developed is shown as Addendum 1.

Sampling

Although the sampling method will eventually be extended to other provinces, the initial study to determine the success of the sampling methods was restricted to the Nelspruit area of Mpumalanga. This area was randomly selected from a list of state veterinary districts in Mpumalanga. A total of 20 (n=20) dip-tanks within the state veterinary district of Nelspruit and 12 (n=12) commercial farms were randomly selected from a list of all the dip-tanks in the district (n=32) Fig.1.

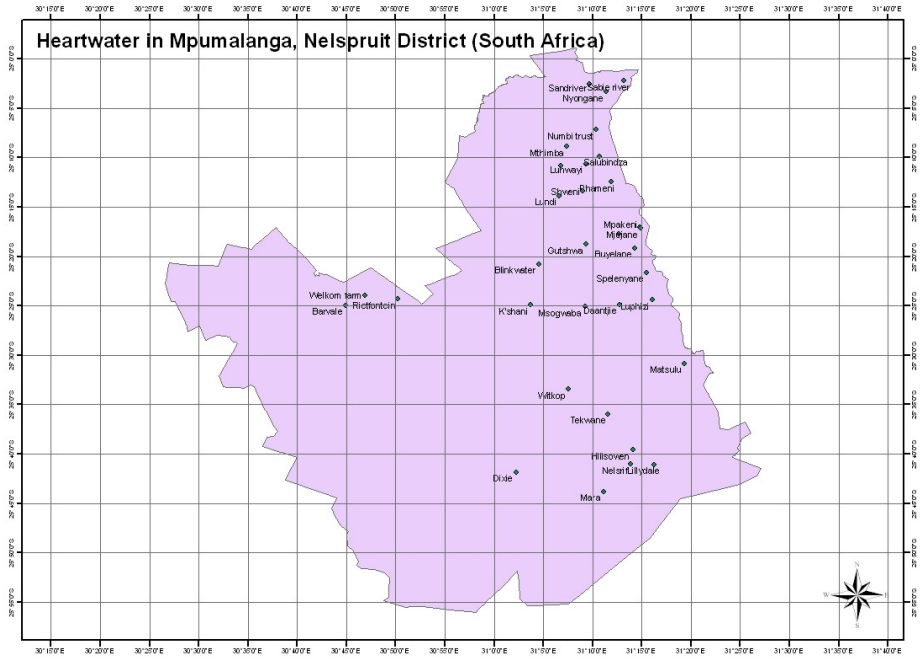

Fig. 1 Study area of the State Veterinary district of Nelspruit from the randomly selected dip-tanks and commercial farms.

From the number originally chosen, (n=13) could not be done because they were inaccessible because of poor road infrastructure and heavy rain and it would have been impossible within the time limits. Therefore the closest dip-tank that was accessible was substituted in each case. This was an important field constraint to the successful implementation of true random sampling. Twenty (n=20) cattle were randomly chosen at each dip-tank. However, two constraints were encountered. Firstly, the week chosen for sampling was characterised by unexpected heavy rain which complicated the handling, identification and sampling of the cattle. Secondly, several farmers did not come to the dip tanks as arranged because of the weather and therefore their cattle were not sampled.

In order to prevent selection bias, therefore, a maximum of five (n=5) animals were randomly chosen from each owner that came as arranged. This constraint was not as serious as it might appear as all the owners grazed their cattle in the communal areas around the dip-tanks and therefore the herds mingled.

Random collection of five (5) ticks was done on each animal sampled (sampling unit 3). However, this was also a problem as certain animals seemed to be much more resistant to ticks and no *A. hebraeum* ticks were found on them. Others in the herd had very high tick burdens, in contrast. The AHT suggested that this discrepancy may also be due to the fact that owners do not always bring their animals to be dipped and sometimes only bring animals close-by to the dip-tank or only bring animals that have heavy tick burdens to be dipped.

DISCUSSION

Ten AHT from the Nelspruit state veterinary district were successfully trained to collect blood and tick samples from livestock and the correct handling and dispatch of samples in line with the Dangerous Goods Packaging regulations as per UN class 6.2, when transported by road. A total of 640 (20 animals per dip-tank) blood samples and only 109 tick samples were collected from dip-tanks and commercial farms. During this process a situational appraisal was done with AHT and also animal owners. Existing ARC-OVI extension material was found to be suitable for training farmers in the recognition of heartwater and understanding of its importance. ARC-OVI also has preserved samples and charts available for the identification of *A. hebraeum* in all its stages.

However, extension materials for training AHT in the correct methods of handling animals, collecting and dispatching samples were not available. Cameron (1999) suggests that a great deal of attention be paid to this, as it can be a major constraint to successful surveillance of animal diseases. Consequently, the information obtained during the training of the 10 AHT's was used to develop suitable extension material, following the method developed by McCrindle (Addendum 1).

CONCLUSIONS

Although many scientific surveys and publications describe the use of "random samples", field experience has shown that truly randomised samples probably only exist on station or in the laboratory. In the field there are many variables that can interfere. In this case the weather, time constraints, human behaviour (farmers being fairly erratic in bringing their animals to the dip-tank) and animal behaviour (some animals were very much wilder than others) were constraints to true random sampling. Variability within animals also had an effect on random sampling of the ticks in the third stratum of the cluster sampling.

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BETTER DIAGNOSIS OF BOVINE TRYPANOSOMOSIS THROUGH PEN-SIDE TESTS FOR ANAEMIA

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ABSTRACT

African Animal trypanosomosis (AAT) is considered the most important cattle disease in sub-Saharan Africa but field diagnosis is often problematic as the most common clinical signs are not pathognomonic. Improved diagnosis is needed to reduce inappropriate treatments while ensuring timely treatment of sick animals. A study in West Africa investigated the utility of the presence of anaemia for detection of trypanosomosis. A total of 20 772 blood tests were carried out in cattle from 121 villages in three countries. The average Packed Cell Volume (PCV) of positive cattle was 23% versus 28% for negative cattle. Anaemia (PCV<24%) was a reasonably accurate indicator of trypanosomosis in the study area, the sensitivity was 56% and the specificity 80%. Of a population of 898 animals suspected to be sick with trypanosomosis, 88% had a PCV of less than 28% and the diagnostic odds ratio of anaemia was 4.2. Anaemia was the most reliable of seven diagnostic signs tested (emaciation, staring coat, lymphadenopathy, fever, lacrimation and salivary or nasal discharge), so, in a further investigation, pen-side tests for anaemia were evaluated.
Two methods of anaemia detection in cattle were assessed: a colour chart developed for anaemia detection by visual inspection of conjunctival membranes in sheep (FAMACHA©) and the Haemoglobin Colour Scale (HbCS) developed for assessing haemoglobin levels by comparing the colour of blood drops on filter paper with colour standards, and used in human patients. In a population of cattle suspected by owners to be sick with trypanosomosis (n=898) the average FAMACHA© and HbCS score differed significantly (p=0.000) between anaemic and non-anaemic cattle. The sensitivity of the HbCS test was 56% and the specificity was 77%, while the sensitivity of the FAMACHA© test was 95% and the specificity was 22%. The higher sensitivity but lower specificity suggests the FAMACHA© may be useful as a screening test and the HbCS as a confirmatory test. The two tests were also evaluated in cattle randomly selected from the village herd. Using cut-off points to optimise test performance the HbCS test had a sensitivity of 81% and a specificity of 62% (n=505 cattle), while the FAMACHA© had a sensitivity of 92% and a specificity of 30% (n=298 cattle).

INTRODUCTION

Anaemia occurs when the total volume of red blood cells (and/or the amount of haemoglobin in these cells) is reduced below normal values, as defined by measurements in healthy populations. Anaemia is a symptom of many cattle diseases including malnutrition and infection with internal and external parasites. It is considered one of the most reliable signs of trypanosomosis (Uilenberg, 1998) which in many parts of Africa is the most important disease in cattle. In West Africa, there is a strong correlation between trypanosomosis prevalence and herd packed cell volume (PCV) indicating the importance of trypanosomosis in the aetiology of anaemia.

There are two approaches to anaemia testing. The first is individual testing and in the veterinary context this is usually carried out when an animal is suspected to be sick. The second is population screening to assess overall herd health (Bauer et al., 1999) or to detect animals requiring treatment in the herd or flock. These different approaches require different test characteristics; where anaemia tests are used to monitor the effectiveness of interventions then an accurate and reliable test is

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needed, where herd screening is carried out in order to detect animals requiring treatment, then cheapness, ease of use and sensitivity are important; while diagnosis of individual animals requires a specific and accurate pen-side test. Resource constraints in developing countries place imply additional requirements. Tests should be cheap, easily available, simple to use and robust under adverse environmental conditions.

Anaemia detection tests may be qualitative or quantitative, with the later being more accurate but also more expensive and complicated. Qualitative tests include clinical examination, filter paper colour comparison tests and the copper sulphate specific gravity test. Quantitative methods include centrifugation, haemoglobinometers, chemical and photometric tests.

The simplest test is visual inspection of mucous membranes and skin. This is non-invasive and in-expensive but very subjective, and prone to inaccuracy especially when examiners are not experienced. In human patients, this method is better at detecting moderate to severe anaemia than mild anaemia, and checking multiple sites improves accuracy. A colour conjunctival chart can also improve accuracy in some settings: with this, sensitivity generally ranges from 16 to 38% and can be as high as 68% with experienced examiners, while specificity ranges from 70 to 100% (Robinett et al., 1996). In the veterinary context, a colour chart has been developed for detection of anaemia in sheep; this has also been applied to anaemia detection in goats. The test consists of a laminated coloured chart with five colour categories showing worsening degrees of anaemia; these colours are compared to sheep conjunctivae. Use of this test has been shown to improve treatment quality and herd health and reduce drug costs (Van Wyk & Bath, 2002). While sensitivity is high, the predictive value of positive tests tends to be low (Kaplan et al., 2004).

Tests for anaemia detection based on the colour of blood on filter paper were developed at the end of the nineteenth century. Early tests were unreliable but in the last decade modern technologies were used to develop a more robust test and with World Health Organisation support a commercial kit became available in 2001 (WHO, 2004). A capillary blood spot collected directly on filter (chromatography) paper is compared to a printed set of colour standards measured in increments of two grams haemoglobin per dl. This method is inexpensive (less than $0.01 per test), simple, rapid and portable, and some evaluations have been very positive (Ingram and Lewis, 2000). However, size and thickness of blood spot, temperature, and humidity all affect drying time, which, in turn, affects colour, while background lighting and observer effects may also result in bias (Palmer, 2002).

In veterinary research in Africa, the most widely used quantitative test for anaemia is micro-centrifugation. Whole blood is collected in a micro haematocrit tube and centrifuged at sufficient speed (7000-9000 revolutions per minute) and for sufficient time to pack red blood cells into a mass measured on a reader as a percentage of total blood volume.

A study in West Africa investigated firstly the usefulness of anaemia in the detection of trypanosomosis. Next, two methods of anaemia detection were assessed in cattle believed by farmers to be sick with trypanosomosis and in cattle randomly selected from the general population: FAMACHA©, a colour chart developed for anaemia detection in sheep (Bath, 1996) and the Haemoglobin Colour Scale (HbCS) filter paper test developed for assessing anaemia in human patients (Scott & Lewis, 1995).

MATERIAL AND METHODS

Data from a series of surveys covering 20 772 tests in 121 villages in three countries were used to assess the usefulness of anaemia as a predictor of trypanosomosis. Blood sampling was carried out by jugular or caudal venipuncture using EDTA or heparin coated vacutainers; samples were placed on ice, and analysed within six hours. To determine packed cell volume (PCV), blood in microhaematocrit capillary tubes was centrifuged at 8000g for five minutes. PCV was read using a Hawkesley micro-haematocrit reader (Hawkesley, Lancing, UK). Trypanosome infection was detected using the Buffy Coat Technique (BCT) (Murray et al., 1977). Species of trypanosome were

identified by morphology and movement and the level of parasitaemia was recorded (Paris et al., 1982). Anaemia was defined as a Packed Cell Volume of less than 24% (Radostits et al., 2000). The FAMACHA© Chart and Haemoglobin Colour Scale were tested in a population of cattle believed by their owners to be sick with trypanosomosis. A standardised clinical examination was carried out on all sick animals, including recording temperature, examination of lymph nodes, measuring girth, assessing body condition and recording clinical signs. Blood was sampled, PCV measured and BCT examined as described above. FAMACHA© (n=891) and HbCS tests (n=503) were carried out by two veterinarians according to the manufacturer's instructions, after an initial practice session. The test results were recorded before the PCV was analysed (i.e. testing was blind). For the FAMACHA© test, animals with a score of 4 and 5 were graded as positive, those with 3 as doubtful and those with a score of 1 or 2 as negative. In the HbCS test, animals with a reading of less than 9 g/dl were graded as positive (normal cattle haemoglobin range is 9 to14 g/dl). The FAMACHA© and HbCS tests were also assessed separately in two populations of cattle (n=298 and n=505 respectively) randomly selected from the same geographic area (Kénédougou, Mali and Burkina Faso).

RESULTS

Usefulness of anaemia in diagnosis of trypanosomosis

Anaemia was a relatively sensitive and specific test for trypanosomosis (Table 1). The average Packed Cell Volume (PCV) of positive cattle was 23.1% (n=1395 cattle) versus 27.8% for negative cattle (n=19 377). In the population studied, the sensitivity of the test was 56.2% and the specificity was 79.5%. The area under the Receiver Operating Curve (ROC) gives an overall measure of test accuracy; the value of 0.68 implies a moderately accurate test. Anaemia was most useful in the detection of *Trypanosoma congolense* infections, somewhat useful in the diagnosis of *T. vivax* and less useful in the diagnosis of *T. brucei*.

Table 1 Test performance of anaemia as a predictor of trypanosomosis (prevalence 6.7%)

	Trypanosomosis	*T. congolense*	*T. vivax*	*T. brucei*
Sensitivity	56.20%	62.80%	46.7	24.5
Specificity	79.50%	79%	77.7	77.1
ROC area	0.679	0.709	0.622	0.508
Likelihood ratio (+)	2.75	2.98	2.09	1.07
Likelihood ratio (-)	0.551	0.471	0.686	0.978
Odds ratio	4.99	6.33	3.05	1.1

There was a wide variation in trypanosomosis prevalence in the villages studied from 0% to more than 50%. Positive and negative test predictive values depend on the prevalence of disease in a population. At a trypanosomosis prevalence of 30%, an anaemic animal had a greater than 50% likelihood of being parasitologically positive for trypanosomosis, while at a prevalence of 2%, the likelihood was only around 5%. Conversely, at a low prevalence of trypanosomosis the likelihood of a non-anaemic animal being negative for trypanosomosis was nearly 99% while at a prevalence of 30% as much as 20% of non-anaemic animals were positive. Table 2 shows the test performance of anaemia as a predictor of trypanosomosis at different disease trypanosomosis prevalences.

Table 2 Test performance of anaemia as a predictor of trypanosomosis at different prevalences

Trypanosomosis prevalence	6.7%	2%	5%	10%	20%	30%
Positive predictive value	16.50%	5.31%	12.60%	23.40%	40.70%	54.10%
Negative predictive value	96.20%	98.90%	97.20%	94.20%	87.90%	80.90%
Pre-test odds		0.0204	0.0526	0.111	0.25	0.429

Post-test odds (+)	0.0561	0.145	0.305	0.687	1.18
Post-test odds (-)	0.0112	0.029	0.0612	0.138	0.236

Usefulness of anaemia compared to other diagnostic signs

Anaemia was the most useful sign for detecting trypanosomosis with an Odds Ratio of 4.2; more than four times as many anaemic cattle test positive for trypanosomosis than non-anaemic. The only other signs tested which had significant Odds Ratio (p=0.05) were pyrexia (OR=2.0), lacrimation (OR=1.7) and enlargement of superficial lymph nodes (see Table 3).

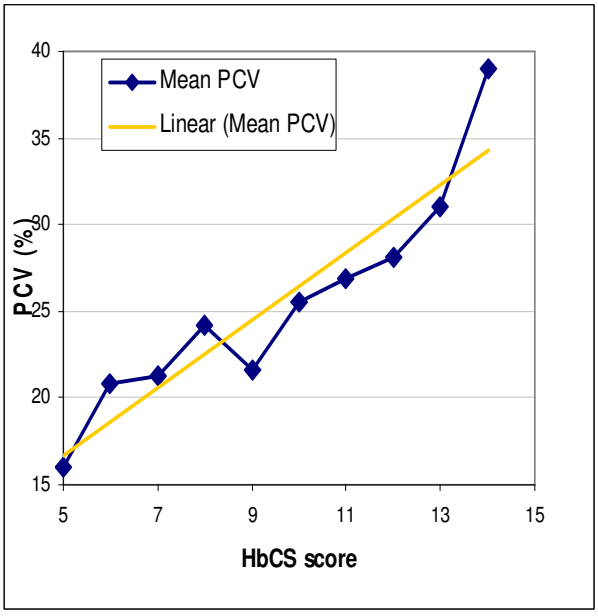
Table 3 Usefulness of different clinical signs as predictors of trypanosomosis

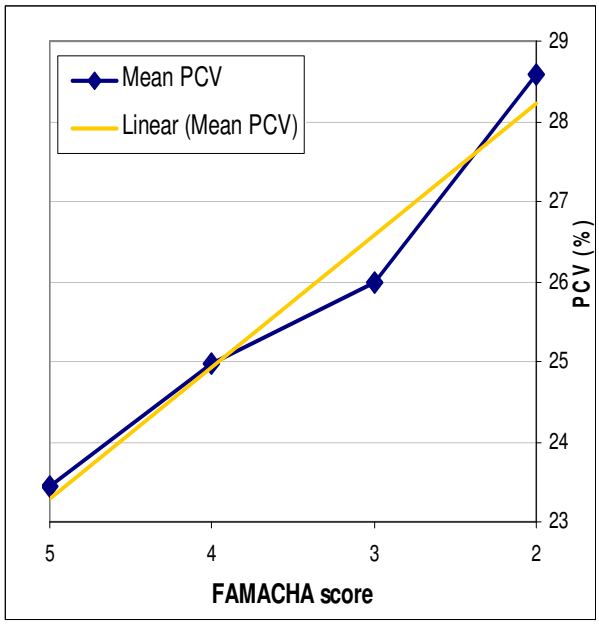
Clinical signs	% with sign	Odds ratio	95% CI for OR	p=
Salivary/nasal discharge	10.7	1.3	0.56 to 2.83	0.445
Emaciation	64.0	1.2	0.77 to 1.88	0.402
Staring coat	67.8	0.8	0.53 to 1.25	0.306
Lacrimation	33.0	1.7	0.96 to 2.83	0.050
Enlarged lymph nodes	59.6	1.6	0.96 to 2.49	0.043
Temperature (>39.4)	17.9	2.0	1.31 to 3.07	0.001
Anaemia (PCV<24%)	35.7	4.2	2.62 to 6.72	0.000

Pen-side tests for detection of anaemia in sick cattle

There was an approximately linear relation between FAMACHA© and HbCS score and PCV (Figure 1, a linear trend line has been added). Cattle negative for anaemia according to the HbCS test has an average PCV of 26.5% significantly higher (p=0.000) than cattle positive on the test, whose average PCV was 22.0 (t test). Similarly, the PCV of cattle negative for anaemia according to the FAMACHA© test had a significantly (p=0.000) higher PCV (28.6%), than positive animals (24.9%).

Figure 1 Relation between HbCS and FAMACHA© scores and average PCV





In the study population, the HbCS test for anaemia had a sensitivity of 56.3% and a specificity of 76.9%. At the prevalence of anaemia found among sick cattle in this area, the positive predictive value (PPV) or probability of an animal which tests positive actually having the disease was 61%,

while the negative predictive value or likelihood of an animal which tests negative not having the disease was 72% (NPV). The test performed better at the detection of severe anaemia (PCV <21%); for detection of severe anaemia the sensitivity was 68.3% and the specificity 72.0%.
The FAMACHA© test had a sensitivity of 95.2% and a specificity of 22.4%, with a PPV of 40% and a NPV of 90%; for severe anaemia the sensitivity was 100% and the specificity was 19.0%.
The performance of both tests improved as the prevalence of anaemia increased. Table 4 summarises the performances of the HbCS and FAMACHA© tests in detecting anaemia (PCV <24) at different prevalences and table 5 shows the performance in detecting severe anaemia (PCV <21).

Table 4 Performance of HbCS and FAMACHA© tests in the detection of anaemia

	HbCS test			FAMACHA© test		
Prevalence of anaemia	39%	95% CI		35%	95% CI	
Sensitivity	56.30%	49.10%	63.40%	95.20%	90.40%	98.10%
Specificity	76.90%	71.70%	81.50%	22.40%	17.60%	27.90%
ROC area	0.666	0.624	0.708	0.588	0.558	0.618
Likelihood ratio (+)	2.44	1.92	3.09	1.23	1.14	1.32
Likelihood ratio (-)	0.568	0.479	0.673	0.214	0.1	0.455
Odds ratio	4.29	2.92	6.31	5.74	2.6	12.7
Positive predictive value	61.00%	53.50%	68.10%	39.70%	34.60%	45.10%
Negative predictive value	73.30%	68.10%	78%	89.70%	79.90%	95.80%

Table 5 Performance of HbCS and FAMACHA© tests in the detection of severe anaemia (PCV <21%)

	HbCS test			FAMACHA© test		
Prevalence of anaemia	20%	95% CI		14.00%	95% CI	
Sensitivity	68.30%	58.30%	77.20%	100%	93.70%	100%
Specificity	72%	67.30%	76.30%	19%	14.90%	23.30%
ROC area	0.701	0.651	0.752	0.594	0.574	0.614
Positive predictive value	37.90%	30.80%	45.40%	16.30%	12.60%	20.60%
Negative predictive value	90.10%	86.30%	93.10%	100%	94.70%	100%

Testing for anaemia in randomly selected cattle

Using the criterion of a score of less than 9 as indicative of anaemia, the HbCS test was highly specific but not very sensitive at detecting anaemia in randomly selected cattle. Choosing a higher cut-off point increased the sensitivity, without important reduction in specificity. Table 6 shows the effect of changing the cut-off on test performance. Greatest overall accuracy is obtained using a cut-off point of 11; if high sensitivity is desired a cut-off of 12 is most useful.
Using the criterion of a score of 4 or 5 being indicative of anaemia the FAMACHA© test was highly sensitive but not very specific. A higher cut-off point (5 considered indicative of anaemia) improved overall test accuracy but decreased sensitivity (Table 6).
Both tests had higher specificity, sensitivity and accuracy when used for detecting anaemia in randomly selected cattle than in detecting anaemia in cattle believed to be sick.

Table 6 Test performance of HbCS and FAMACHA© using different cut-offs in a population of randomly selected cattle

	HbCS test	FAMACHA©
Prevalence of anaemia	18%	27%

Cut-off	9	10	11	12	4	5
Sensitivity 95% CI	15.70%	25.80%	59.60% (48.6-69.8)	80.90%	92.40% (84.2-97.2)	57%
Specificity 95% CI	100%	98.80%	89.90% (86.3-92.4)	61.50%	30.30% (24.3-36.8)	88.50%
ROC area 95% CI	0.579	0.623	0.746 (0.693-0.799)	0.712	0.613 (0.571-0.656)	0.727
Likelihood ratio (+)	.	21.5	5.9	2.1	1.33	4.97
Likelihood ratio (-)	0.843	0.751	0.45	0.31	0.251	0.486
Odds ratio	.	28.6	13.1	6.78	5.28	10.2
Positive predictive value 95% CI	100%	82.10%	55.80% (44.7-65.4)	31%	32.40% (26.4-39.0)	64.30%
Negative predictive value 95% CI	84.70%	86.20%	91.20% (88.0-93.8)	93.80%	91.70% (82.7-96.9)	85%

DISCUSSION

The study supported the finding of other authors that anaemia was a useful sign for the diagnosis of trypanosomosis in cattle, and that *T. congolense* is associated with a more severe anaemia than *T. vivax*. The method of trypanosomosis detection (buffy coat examination after centrifugation) used in this study is relatively insensitive in the detection of disease (Gall et al., 2004) and so this study probably under-estimated the usefulness of anaemia as a predictor of trypanosomosis.

Two pen-side tests for the detection of anaemia in cattle were evaluated. The HbCS test was developed for detection of anaemia in human patients. A recent review considered 14 studies on HbCS diagnostic accuracy for detection of anaemia (Critchley and Bates, 2005). This found that sensitivity for detecting anaemia was high in most of the studies (75–97%); specificity was generally lower (41–98%), with results from field-based studies generally worse than those from laboratory studies. In the study carried out in cattle believed to be sick we found high sensitivity and specificity (70%) for detection of moderate to severe anaemia in cattle; however, the test was less sensitive for the detection of mild anaemia. In the study carried out in randomly selected cattle good sensitivity and specificity were attained.

Studies of the FAMACHA© test in goats and sheep showed a test sensitivity of 55 to 100%, and a specificity of 30 to 90% (Vatta et al., 2001; Kaplan et al., 2004). In the study in the population of sick animals sensitivity was good (>90%) but specificity lower (20 to 30%) than this. However, the test was highly sensitive in the detection of severe anaemia (100%). In the population of healthy animals the test showed high sensitivity and moderate specificity when using a low cut-off. This test performance would be desirable where the effects of disease are serious, the costs of treatment moderate and there is little evidence of over-treatment. Using a high cut-off resulted in a test of moderate sensitivity and high specificity; this would be desirable when the effects of disease are less serious (e.g. predominantly trypanotolerant cattle) and/or there is concern about over-treatment and emergence of drug resistance.

The HbCS had better overall performance (as reflected in the higher AUC) than the FAMACHA© in detecting both anaemia and severe anaemia in animals believed by their owners to be sick. The FAMACHA© test had better overall performance in the detection of anaemic cattle in the general population. However, unlike the tests in sick animals, these tests were carried out in two different populations, and there was a higher prevalence of anaemia in the population in which the FAMACHA© test was evaluated, making comparisons between the two tests difficult.

Both tests were rapid, simple, and easy to perform with minimal training. The HbCS test requires a drop of whole blood and this makes it more suitable for use by para-veterinarians and veterinarians; the FAMACHA© chart is non-invasive and we successfully trained illiterate farmers in use of colour charts (results not discussed here). However, the written material accompanying

both tests is not designed for use in cattle (but humans and sheep respectively) and would need modification to facilitate its use in this species. Translations into the language of the users would also be desirable; the HbCS test literature is in English and French and the FAMACHA© in English only.

This evaluation suggested that both tests may be useful in improving diagnosis of anaemia in cattle. The greater sensitivity of the FAMACHA© test in detecting disease suggests it may be useful as a screening test, and where there is less concern with excessive treatments while the greater specificity of the HbCS test is useful in confirming (ruling-in) a diagnosis and where there is more concern about over-treatment. The choice of cut-off point depends on whether the test is being carried out on a herd basis or on animals already showing signs of illness and on the relative costs of treatment (including the externality of drug resistance) and non-treatment.

CONCLUSION

In tsetse-infested sub-humid, West Africa, anaemia is a useful diagnostic sign of trypanosomosis, Pen-side anaemia tests are cheap and easy to use and can improve the diagnosis of anaemia; hence they are likely to improve the treatment of trypanosomosis, helping ensure that sick animals receive the treatment they need while reducing the number of un-necessary treatments that waste scarce resources and foster drug resistance. While this initial investigation indicated accuracy of tests was high enough to be useful as field tools, further investigation is needed into clinical outcomes, costs and benefits of using pen-side anaemia tests in cattle where trypanosomosis is endemic.

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AN OUTBREAK OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN OSTRICHES IN SOUTH AFRICA IN 2004

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ABSTRACT

An outbreak of Highly Pathogenic Avian Influenza (HPAI) in ostriches caused by type A Avian Influenza Virus subtype H5N2 (AI H5N2) occurred in the Eastern Cape Province of South Africa in July 2004. The outbreak became apparent when abnormally high ostrich mortalities were reported from a specific farm in the Somerset East area. Mass ostrich mortalities were preceded by a 3 month period characterized by low grade infection and mortality not exceeding 5%. Intensive surveillance was undertaken on 190 ostrich farms and holdings in the province out of which 19 farms were either infected or shown to have evidence of exposure to Avian Influenza Virus (AIV).

Data derived from 5 HPAI infected farms revealed that the morbidity ranged from 25% to 90% and the mortality from 6.6% to 44.5%. A total of 7232 ostriches or 32.2% of the population on the five farms were found to have died between June and mid-August 2004. The clinical symptoms and pathological lesions observed varied from farm to farm but were typical of the disease. In addition, fungal granulomas were prevalent in the lungs and air sacs. Since disease was not always apparent in seropositive flocks, it is possible that clinical disease only became manifest under field conditions due to secondary bacterial or fungal infections and/or following managemental stress factors.

Serum, tissue, cloacal and tracheal swab samples were collected from ostriches on various farms and subjected to tests for AIV. Blood samples from 186 farms were tested using the Haemagglutination Inhibition (HI) test and 17 were found to be positive for AIV H5. Virus isolation was undertaken on tissues obtained from ten farms that exhibited ostrich mortality and only material from 2 farms yielded positive virus isolates. Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) assays and cycle-sequencing of the viral genomes indicated that two of the viral isolates were both a HPAI H5N2 subtype. RT-PCR done on swabs obtained from 136 farms yielded positive results for presence of AIV on 3 of the farms. Samples from seven farms selected on the basis of a high exposure risk profile or suspicious RT-PCR test findings were subjected to Nucleic Acid Sequence-based Amplification (NASBA), an isothermal amplification transcriptional procedure, and 4 were found to be positive for AIV H5. The disease was controlled by stamping out and altogether 25945 ostriches were culled on 37 infected and in contact farms.

INTRODUCTION

Notifiable Avian Influenza (NAI) caused by certain type A influenza viruses, has in the recent past caused epidemics affecting primarily poultry in Italy in 1999 – 2000, Holland in 2003, Canada in 2004 and Asia in 2003 – 2004 (Capua, 2006). The disease was first diagnosed in young ostriches in South Africa in 1991 (Allwright et. el., 1991) and was shown to be caused by a low pathogenic (LPAI) H7N1 subtype virus. Other isolations of mainly LPAI subtypes from ostriches were made in subsequent years. In addition, numerous virus isolations have been made from wild water fowl in South Africa (Allwright et.al., 1993; Abolnik et al. 2006). Indications of virus prevalence in wild

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waterfowl in South Africa was seen in a survey undertaken in 1998 in which eight LPAI H10N9 subtype isolates were made from organ pools of seronegative birds (Pfitzer et. al. 2000). However, none of the isolates were linked directly to any AI outbreak in poultry in the country.

AI viruses apparently vary considerably in their ability to infect and cause disease in different host species (Swayne and Halvorson, 2003). Until the recent outbreak of AIV H5N2, all AIV isolations made from ostriches and chickens in South Africa between 1991 and 2004 were of low pathogenicity (Allwright et.al., 1993; C Abolnik et. al, 2006). Even the AI H5N2 virus isolated from ostriches in Zimbabwe in 1995 (Huchzermeyer, 2002) was an LPAI virus. All this contrasts with the outbreak of AI H5N2 in ostriches in the Eastern Cape Province of South Africa described here, which caused significant morbidity and mortality on several farms. An overview of the findings and observations from this outbreak of ratite HPAI is presented.

MATERIALS AND METHODS

All farms within the Eastern Cape Province were sampled in terms of a specific protocol which indicated that 29 blood samples per epidemiological unit were to be collected. Tracheal and cloacal swab samples were collected simultaneously. Tissue samples from fresh post mortem specimen were taken for virus isolation or detection. Tissue samples were also collected in 10% formalin for histopathological analysis. Samples were tested at the OVI, Allerton PVL and some at Stellenbosch PVL . Detection and isolation of AIV from the index farm was done at Stellenbosch PVL and Avimune private laboratory.

Serological techniques used were the Agar Gel Immunodiffusion (AGID) test for detection of antibodies to nucleocapsid and matrix antigens specific for influenza A type virus, the Haemagglutination Inhibition (HI) test for routine detection of the H5 subtype using the H5N2 virus derived from the primary isolation as the antigen. The Enzyme-linked Immunosorbent Assay (ELISA) was used to detect antibodies to influenza A type specific antigens in chickens only.

Virus was detected using RT-PCR carried out on allantoic fluid, tissues and swabs. Nucleic Acid Sequence-based Amplification (NASBA) was performed at OVI on selected samples considered to be of high risk. Extracts from tissues and from swabs found to be PCR positive were also inoculated into specific pathogen free (SPF) embryonated chicken eggs for virus isolation. Haemagglutination (HA) or AGID was performed on allantoic fluid following any successful virus growth in SPF embryonated chicken eggs.

RESULTS

A total number of 32 581 samples were collected from 286 farms of which 29 586 were derived from ostriches on 190 farms. Nineteen of the ostrich farms tested positive for H5 AIV, 17 were positive on serology, 6 were positive for viral RNA on RT-PCR and NASBA tests on swabs and virus was isolated from 2 farms. Ostriches were the only species positive for H5 AIV in the Eastern Cape (Table 2). No clinical disease or AI ELISA positive cases were detected in chickens. Ostriches on ten out of nineteen AIV seropositive ostrich farms exhibited clinical infection with the disease. The clinical signs observed on the infected farms included sudden deaths, depression, anorexia, swollen head and eyes, suppurative ocular discharge, conjunctivitis with encrustation, sinusitis, wet cough, green fluorescent urine, respiratory distress evidenced by gaping, and a complete stop in egg production. Morbidity on these farms ranged from 25 to 100% and mortality from 6 to 44% (Table 1)

Table 1 Mortality and morbidity on 5 ostrich farms infected with HPAI

Farm	Population	Number sick	Number Dead	Percent Mortality	Remarks
Endor	8131	>90%*	3621	44.5%	Aspergillus lesions present
Resolution	6500	75%	2177	33.5%[a]	Aspergillus lesions present
Glentana	4984	75%*	1239	24.9%	Aspergillus lesions present
Cliff Royal	580	25%*	45	7.8%	
Draaihoek	2266	25%)	150	6.6%	Aspergillus lesions present
Totals	22461		7232	32.2%	Average mortality

* - Estimated morbidity
a - Mortalities for Resolution farm determined as the difference between the original population and final number of live birds culled

Four of the 5 farms also had dead ostriches with granulomatous air sacculitis caused by *Aspergillus fumigatus* (Table 1). Mortality was highest in young ostriches less than 6 months (Table 2). Low mortality in breeders was reported only from the index farm of the HPAI outbreak.

Table 2 Age group related ostrich mortality at 2 HPAI infected farms

Age Group	Mortality rates % Glentana	Endor
0-40 kg <6 months	48.8	77.2
40-70 kg 6-8 months	36.2	35.8
70-90 kg > 8 months	4.7	
Breeders	0.0	8.4

Ostrich mortality started on the farm Endor in March 2004, escalated in May followed by an exponential rise from June until culling of ostriches was completed in mid August 2004 (Fig.1). Moderate ostrich mortalities occurred at the farm Glentana at the beginning of June, also rising substantially until all ostriches were culled on the 19th of August 2004. Mortality in Draaihoek started in August 2004 and 150 ostriches died within 2 weeks just before completion of culling on the farm on 16th of August 2004.

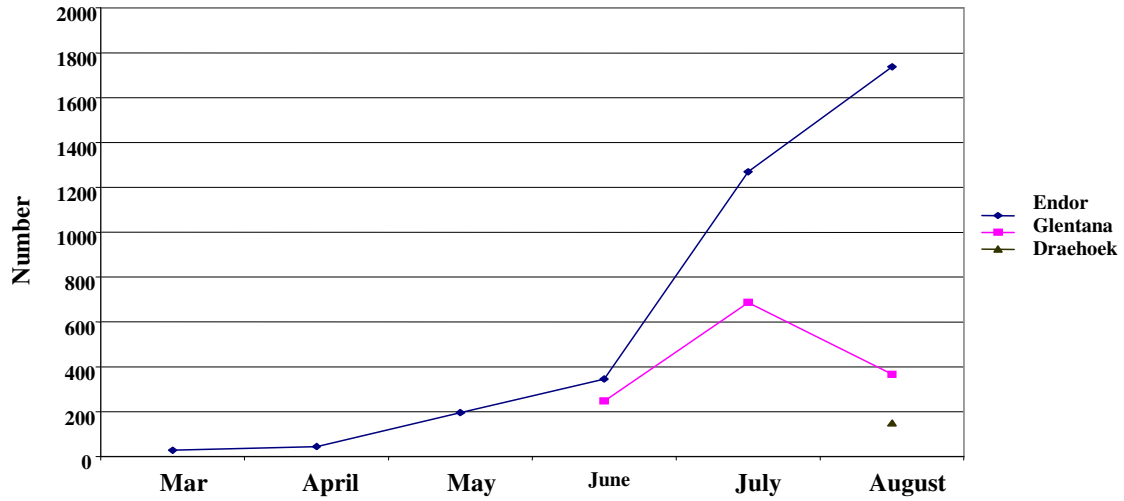

Fig1 . Graph showing the trend in ostrich mortalities on 3 of the AI Positive Farms in Somerset East in 2004

Pathological lesions included oedema and fibrin deposits found in the retropharyngeal and retro-orbital areas of the swollen ostrich heads. Fibrino-suppurative deposits were present in the air sacs and lungs of some ostrich carcasses. Liver degeneration and multifocal to diffuse necrosis and in one instance fibrosis was observed. Petechial and ecchymotic haemorrhages were observed on the epicardium, airsacs, intestines and pancreas. Pulmonary congestion and frequently oozing blood on cut surface was often encountered. The kidneys were usually enlarged with prominent and degenerate tubules. Secondary, or possibly even primary fungal microgranulomas shown to be due to *Aspergillus fumigatus* , were found extensively in the air sacs of many ostrich carcasses from 4 of the 5 farms (Table 1). Histologically the liver had multifocal to coalescing areas of necrosis with moderate infiltration of neutrophils, macrophages, lymphocytes and plasma cells; the spleen exhibited multifocal to coalescing areas of necrosis with fibrin thrombosis; the kidney showed moderate diffuse congestion; the lung had multifocal small granulomas in the parabronchial walls consisting of neutrophils, mononuclear cells and small fine bacterial bacilli; air sac granulomas consisted of fibrin, neutrophils and fine bacterial bacilli; small intestines revealed extensive focal necrosis of the lamina propia with numerous neutrophil and scanty mononuclear cell infiltration into the submucosa and muscle.

Table 3 Results of HPAI testing of various farms in the Eastern Cape.

Species	Proportion of positive farms				
	HI	ELISA	RT- PCR	NASBA	Virus Isolation
Ostrich	17/186		3/139	4/7	2/10
Chicken	0/20	0/96	0/5		
Turkey	0/5				0/1
Duck	0/8				
Goose	0/12		0/7		
Wild Goose	0/4		0/7		
Porcine	0/16				
Parrot			0/2		
Pigeon	0/12				
Blue crane			0/1		
Guinea Fowl	0/1				
Finch			0/1		

Out of 186 ostrich farms tested serologically, 17 were positive for H5 AIV using the HI test. A number of farms with other AI risk species such as pigs, wild and domestic geese were also tested using HI but were all found to be negative (Table 3). The 96 farms/holdings and/or owners of chickens tested for AI using ELISA were found to be negative but positive results were found for 11 of them. The ELISA positive chicken samples tested negative on the HI test and they were considered to be false positives.

The antibody titres on the HI positive farms ranged from 4 to 11 (dilutions of 1:16 to 1:2048). All seropositive ostriches were aged 6-12 months including some of the breeding ostriches. AGID test results for HI positive farms revealed positive results for 5 farms (Table 4). Two domestic geese from a farm within the AI surveillance zone tested positive for AI on AGID test but were negative on the HI test.

Of the139 ostrich farms tested for AIV RNA using RT-PCR, 3 were positive and 3 gave suspect positive results.. Out of 7 farms selected for testing using NASBA, 4 were positive for AIV RNA including three which had tested suspect positive using RT_PCR. Virus was isolated from the index farm and from a second farm out of 10 ostrich farms tested (Tables 3 & 4) and was then characterised as HPAI H5N2 according to sequence data obtained at the OVI. These findings were confirmed at VLA, Weybridge. In addition, an intravenous pathogenicity test (IVPI) was carried out there and an index value of 0.63 was obtained, suggesting low pathogenicity for chickens. Following subsequent passages in embryonated eggs, a higher IVPI value of 1.19 was obtained. After a second passage and IVPI test, an index of 2.73 was recorded and the virulent cleavage site was revealed following nucleic acid sequencing.

Table 4 Summary of the Serological, viral detection and isolation test results

Farm Name	**HI Sero-positive farms**			**AGID**	**RT_ PCR**		**NASBA**	**Virus Isolation**
	Days to Sero+	Proportion positive	HI Ab Titers		Swabs	Tissues	Swabs	
Farms at the epicentre of the AI outbreak – Endor = index farm – Somerset East area								
Endor	0	8/20				Pos		Pos
Voorspoed 1	0	50/80	4 -11					
Glentana	3	91/128	4 -10					
Draaihoek	11	57/71	4 - 9	Pos				
Grootplaas	20	56/58	4 -7	Neg				
Voorspoed 2	21	5/54		Pos				
Kareekrans	24	53/88	4 - 9	Pos				
Altona	48	9/35	4 - 8			Pos		Pos
Farms located 100-150 km South of the epicenter of the AI outbreak – Grahamstown area								
Resolution	32	231/763	4 - 8	Pos	Pos			
Boskey Dell	137	4/23	N/A		Pos			
Avondale	?	18/18	5 -11					
Salem Park	?	112/189	4 -11	Pos				
Farms located 60-100 km North of the epicenter of the AI outbreak – Graaff Reinet area								
Klipfontein	81	61/334	4 - 7		Pos			
Dalham	82	13/30	4 - 7		Susp		Pos	
Sero positive farms not culled due to inconclusive findings								
Saltpandrift	11	2/100						
Grasmere	34	8/317	4 - 6					
Modderpoort	82	10/106	4 - 5		Susp		Pos	

RT-PCR positive but sero negative farms that were not culled			
Rustig	Neg		Pos*
Belvedere	Neg	Susp	Pos*

* considered false positives - no seroconversion up to the last bleeding 20 weeks later
Titres 4 to 11 = dilutions 1:16 to 1:2048
N/A – titres not available

Ostriches were culled on 37 infected and in-contact farms (table 5). Culling of ostriches was carried out by a combination of electrical and captive bolt stunning and bleeding. The dead ostriches were dumped into six meter deep pits and covered with lime prior to burial with earth. The disease was controlled by culling of 25945 ostriches and the destruction of 6258 ostrich eggs (Table 5). A total of 2812 mainly back-yard chickens were also culled.

Table 5 Basis for culling of ostrich farms

Reason For Culling	**Number of Farms Culled**
Clinical signs, Mortalities, Virus isolated, Seropositive	1
Clinical signs, Mortalities, Virus detected, Seropositive	2
Clinical signs, Mortalities, Seropositive	4
Virus detected, Seropositive	3
Sero- positive	3
Clinical signs and mortality	3
Clinical signs	1
Unable to obtain samples	1
3 km radius	19
Total Number of farms culled	**37**
Ostriches culled	25945
Ostrich Eggs destroyed	6258
Chickens (Backyard)	2812

DISCUSSION

The outbreak of HPAI H5N2 in ostriches in the Eastern Cape province of South Africa in 2004 was the first to cause severe clinical disease and significant mortality in ostriches of all ages in the region. While the H5N2 had been isolated from ostriches on numerous occasions (Allwright et.al., 1993; Olivier 2006; Huchzermeyer, 2002), it was found in the low pathogenic form and caused disease and mortality only in young ostriches (Allwright et. al. 1993).

The observation in this exercise revealed that mortality ranging from 5% to 45% on individual farms occurred only in the ostrich populations. The backyard poultry primarily chickens and a few domestic geese, ducks, turkeys and pigeons were all unaffected and did not show evidence of sero-conversion either. This obviously indicated some sort of variation in the ability of the AI virus to cause disease in specific host species (Swayne and Halvorson, 2003). Mortality was found to be highest in young ostriches of up to 6 months in age but minimal or absent in breeders.

AI H5N2 virus was isolated from only 2 farms and evidence of virus presence was shown on 7 other farms using RT_PCR and NASBA techniques. Two of the farms which tested positive by NASBA test alone did not seroconvert. The significance of this finding is unclear. However, since disease typical of AI was not seen on these farms until the end of the investigations the ostriches on these farms were not culled. Ten of the 17 HI seropositive farms had no evidence of virus presence. This can be explained by the limited duration of virus shedding that is expected in contrast to the

antibody fingerprint that can remain for a considerably longer period after infection. Initial indications were that the AI H5N2 virus affecting ostrich in Somerset East was of low pathogenicity for chickens as there was no evidence of infection or seroconversion in backyard chickens in the same farms harbouring sick ostriches or anywhere else in the contact area of the outbreak. IVPI determinations indicated a low value for chickens initially but rose rapidly on subsequent passages through chickens. The HPAI amino acid sequence found initially was confirmed at VLA Weybridge (Manvell et. el., 2005). Although not shown experimentally to be very virulent for chickens despite its virulent genotype, this virus poses the serious potential of undergoing further adaptations for infection and spread in chickens that would also allow its virulence to become manifest, as seen following subsequent passage through eggs and chicks. In the case of ostriches, however, it could be that the manifestation of high pathogenicity as observed on several outbreak farms was related to concomitant presence of various stress factors.

Granulomas were consistently found in dead ostriches in the majority of farms. *Aspergillus fumigatus* was isolated from these lesions and the histopathological picture confirmed typical aspergillosis pathology. The significance of this finding and the role that this organism may have played in the pathogenesis of the disease that killed ostriches during this outbreak is uncertain. Although signs typical of AI were also consistently evident in all the farms, it is possible that aspergillosis may have exacerbated the clinical picture and aggravated morbidity and mortality on these farms. During the restocking exercise, it was observed that significant ostrich mortalities caused by infection with aspergillus occurred on the restocked farms in the complete absence of AIV infection (Akol unpublished data) While this may suggest that morbidity and mortality was primarily due to aspergillosis with AI perhaps only increasing susceptibility to such infection; some of the AI positive farms with ostrich mortality in the Eastern Cape were not infected with aspergillosis.

The primary source of infection on the index farm remains unknown. Isolation of the virus in water birds in South Africa (Pfitzer et. al. 2000; C Abolnik et. al., 2006) suggests possible introduction by migratory birds. The recent epidemics in Europe (Capua 2006) makes it plausible that this can be seen as a forerunner of what could happen to this region. The internal spread of AIV after its initial introduction would appear to have been due to uncontrolled movement of ostriches, contaminated feed and water as well as via vehicles and people. This was deduced following a survey on farming practices amongst farmers in the region.

In order to fully interpret the seroconversion data, the index farm in this exercise was considered to be the farm of secondary origin for the outbreak. The other farms which tested serologically positive for AI H5 seroconverted some 3 to 137 days thereafter. The exception was two farms located to the south of the index farm whose history of stocking with sick ostriches 6 months earlier; the observed lack of clinical evidence of disease at the time of testing and the very high proportion of HI positive reactors, suggested that the 2 farms may have been infected earlier than those at the epicentre of the HPAI outbreak. The process of culling ostriches to control HPAI was carried out on all farms where virus was isolated as well as on those farms that were positive serologically for H5 using HI tests. All the farms within a 3 km radius of the farm identified for culling were depopulated as well. Farms from which virus was detected but not isolated and which seroconverted for H5 were depopulated in the same manner.

In conclusion, the outbreak of HPAI H5N2 was pathogenic only for ostriches in the Eastern Cape Province. Molecular tests revealed that the virus had a highly pathogenic genotype but IVPI data indicated it to be initially of low pathogenicity for chickens, with a rapid increase following passages through embryonated eggs and chicks. . Mortality following viral infection may have been exacerbated or possibly caused by secondary aspergillosis.The manifestation of disease in AIV infected ostriches appears to be less predictable and might only do so when additional stress factors are present.

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PRELIMINARY NOTES ON THE CLASSICAL SWINE FEVER OUTBREAK IN THE EASTERN CAPE PROVINCE SOUTH AFRICA

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ABSTRACT

Classical Swine Fever (CSF) was last reported in South Africa in 1918. The recent outbreak of the disease was detected in the Eastern Cape Province of South Africa in July 2005 and confirmed as Classical Swine Fever virus (CSFV) by the Onderstepoort Veterinary Institute in early August 2005 using CSFV antigen and antibody ELISA tests on tissues and serum. The Veterinary Laboratories Agency, Weybridge confirmed CSFV in 3 isolates from the Eastern Cape (2005) and 1 isolate from the Western Cape (2004) using RT-PCR and genotyping demonstrated all 4 isolates to be identical and therefore from the same source of infection. The virus was phylogenetically in the same cluster as 2 isolates from China (1998) and Laos (1999). Subsequently, PCR and sequencing have been used to study the outbreak in more detail.

The disease outbreak affected the eastern and central areas of the Eastern Cape Province which practice communal rearing of pigs. However, small and large commercial piggeries in various parts of the province were also infected. The large commercial piggeries belonged to one company and disease was spread by movement of infected pigs between farms of this company. While spread was presumed to have occurred between the communal and commercial systems of pig rearing, no direct causal link was identified either way.

The outbreak of the disease was characterized by variable pig mortality ranging in one affected communal area from 5 to 22% but with up to 100% mortality occurring in individual homesteads. The pigs in the large commercial enterprises were usually culled before large scale mortality ensued. Observations in some rural communities revealed that areas found to have pigs with high antibody titres experienced high pig mortalities two to three months previously but no mortalities were evident at the time of testing. During culling however, most pigs were found to be in poor condition.

The clinical disease symptoms were preceded by a history of anorexia for at least one week before appearance of signs. Some cases died suddenly with no obvious signs, chronic cases were usually sick for an average of a month. Typical CSF symptoms were observed in cases displaying signs of disease. Ascites with sero-sanguinous fluid was frequently encountered in cachexic cases and sometimes pneumonia was present. The number of pigs culled as at the end of February 2005 was 225297.

INTRODUCTION

Classical Swine Fever (CSF) also known as Hog Cholera or European Swine Fever is an acute viral infection of pigs characterized by high morbidity and mortality caused by a *Pestivirus* of the family *Flaviviridae* (Moennig *et al*., 2003). Primarily a disease of North America and Europe, it has now spread to other parts of the world including South America and the Pacific region. The most recent epidemic of CSF occurred in the Netherlands between 1997 and 1998 where millions of pigs were put down in a control measure to eradicate the disease (Elbers *et.al*., 1999). The presence of the CSFV in wild pigs in Europe makes all attempts to eradicate the disease there almost

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impossible. CSF was last reported in South Africa in 1918 (Plowright *et al*., 1994) where it then re-emerged during 2004 in the Western Cape Province of South Africa. By the time it was confirmed, the disease had spread to the neighbouring Eastern Cape Province The outbreak of CSF in South Africa affected pigs in both the commercial farms and communal rural areas. This paper describes preliminary findings on surveillance, diagnosis and control of the disease in the Eastern Cape Province.

MATERIALS AND METHODS

Study area

Initial surveillance was conducted in an area of 30 km radius around the holding at Qolora by sea where the first case of the disease was confirmed. Surveillance was targeted at swill fed farms/holdings where all pigs were reared outdoors. Areas identified as probable risk due to movement of people from the disease focus such as residential areas of farm staff or others linked to infected holdings and commercial piggeries were all investigated. Before culling started, a snap mini census was conducted in all the wards of Centane and in some wards in Dutywa and Nqamakwe (Figure 1). A questionnaire was completed which sought to establish the pig population, morbidity and mortality in these areas which were first affected by CSF.

Clinical, serological and post mortem surveillance for CSF in the entire province was undertaken due to unknown movement of meat from pigs slaughtered informally and sold in the streets of towns within the infected area. Pigs found in all townships within the Eastern Cape Province were especially targeted due to probable infection from meat brought from infected areas.

Sampling strategy

Samples were collected using a specific protocol. A minimum of 10 blood samples (maximum 35) were to be collected per epidemiological unit for serological testing. Fresh tissue specimens (tonsil, spleen, kidney, mesenteric & maxillary or sub-mandibular lymph nodes and distal ilium) and heparinsed blood for virus detection and isolation were collected from dead and sick pigs respectively. Blood samples for serological diagnosis were taken from live and sick pigs and collected in 7 ml plain evacuated tubes.

Culling

Pigs were put down using .22 calibre rifles at commercial farms and by captive bolt stunning and bleeding at communal areas. Sedation with Azaperone (Stressnil, Bayer AH) or Acepromazine (Neurotranq, Virbac) was used during transportation of pigs from collection sites to culling sites in communal areas. Piglets were euthanased at collection points using Sodium Pentobarbitone (Euthanaze, Bayer AH). The abdomens were slit open and the carcasses were buried in 4-6 meter deep pits.

Laboratory assays

Serological tests:

The Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detect antibodies specific for CSFV as well as to detect CSFV antigen in heparinized blood or tissue at the Onderstepoort Veterinary Institute (OVI).

Amplification of genetic material and phylogenetic analysis:

Molecular diagnostics were performed on three virus samples from the Eastern Cape Province and one from the Western Cape Province at the Veterinary Laboratories Agency, Weybridge (VLA). PCR targeting the E2 gene (envelope glycoprotein) was performed using primers E1 *(*5' AGR CCA GAC TGG TGG CCN TAY GA) and E2 *(*TTY ACC ACT TCT GTT CTC A) resulting in a 671 bp product. The PCR cycling conditions were as follows: 35 x (95°C/45sec, 55°C/1 min, 72°C/1min), 1x (72°C/5 min). Sequencing was performed using 2 internal primers (5' TCR WCA

ACC AAY GAG ATA GGG 3') and (5' CAC AGY CCR AAY CCR AAG TCA TC 3') and the phylogram drawn using 190nt.

RESULTS

Area affected by CSF

Results of a mini census conducted in Centane and parts of Dutywa and Nqamakwe are shown in (Table 1). There were 3 to 4 pigs per owner or homestead. The mortality rate in Centane was 19.8% but individual owners or homesteads were found to have lost all their pigs. At Dutywa and Nqamakwe the mortality rate varied from 4.3%-5.8%. Pockets of CSF negative & clinically healthy looking herds of pigs were present within the communal environment.

Table 1 Results of a mini census conducted in Centane, Dutywa and Nqamakwe indicating the pig population and mortalities between July and August 2005.

Town	Total Number of pigs	Number of sick pigs	Number reported dead	% mortality	Number of owners or homestead	Average number of pigs per owner or homestead
Centane – 54 wards	14729	2070	2890	19.6	4434	3.3
Dutywa – 7 of… Wards	2221	161	95	4.3	516	4.3
Nqamakwe – 8 of …wards	1210	22	71	5.8	366	3.0

The first clinical report of the disease was made from Qolora by sea, Centane (28˚25' E 32˚38' S) in Amatole district municipality on 19th July 2005 (Figure 1, map). Blood samples obtained from two pigs with temperatures of 41.1˚C and 41.7˚C respectively and with enlarged tonsils tested serologically negative for CSFV antibody. However, tissue samples obtained from a sow that died in the same homestead on 26th July 2005 were found to be positive for CSFV antigen. Two weeks later CSF was detected in a major breeding commercial Sow unit in Queenstown (Figure 1, map). Three more commercial farms, one in Aliwal North and 2 in Cookhouse became infected in mid August 2005. These piggeries obtained their normal supply of weaned piglets from the sow unit in Queenstown.

During August and prior to implementation of movement control measures, increased informal pig slaughter and sale of pig meat on the streets of Centane especially at taxi ranks was observed.

A total of 25580 samples were tested for CSF antibodies and antigen in the Eastern Cape Province between date and date, 20921 from communal areas and 4413 from commercial farms and small holdings of which 1224 samples from communal areas and 132 from commercial farms were positive for CSFV (Table 2). From communal areas 5% were positive for antibodies and 36% of tissue samples contained virus antigen compared to 1.6% serologically positive animals in commercial farms and 38% positive for viral antigen. Two hundred and fifty six samples from areas of Lesotho bordering Eastern Cape Province were serologically negative for CSFV.

Table 2 Summary of results indicating the number of animals positive on antibodies and CSFV antigen in communal and commercial areas between date and date

	Communal		Commercial		Combined total	
	Total Tested	CSF ELISA Positive	Total Tested	CSF ELISA Positive	Total tested	Total Positive
Serum samples – antibody	20360	1083 (5%)	4156	65 (1.6%)	24516	1148(4.5%)
Blood samples – antigen	177	1 (0.6%)	135	21 (15.5%)	312	22 (7.1%)
Tissues samples – antigen	384	140 (36%)	122	46 (38%)	506	186(36.7%)
Total specimen	20921	1224	4413	132	25580	1356(5.3%)
Lesotho	251 - All Negative					

CSF outbreaks occurred in townships and small holdings around certain towns. Clusters were identified according to district municipality. Cluster 1 located in Chris Hani consisted of Queenstown and Sterkstroom; cluster 2 located in Cacadu involved 2 groups of towns, 2(1) consisted of Cookhouse and Somerset East, cluster 2(2) consisted of Port Elizabeth and Uitenhage; cluster 3 located in Ukhahlamba consisted of Aliwal North, Burgersdorp, Venterstad and Steynsberg; cluster 4 located in Amatole consisted of East London (Figure 1 map; Table 3). Infected commercial farms were present in clusters 1, 2(1) and 3.

Table 3 Numbers of infected commercial farms, townships and small holdings between August 2005 and January 2006

District Municipality	Towns	Infected farms	Infected Townships	Infected small holdings
Cluster 1 Chris Hani	**Queenstown*** Sterkstroom	1	4	1
Cluster 2 Cacadu 1	**Cookhouse*** Somerset East	2	0	1
Cacadu 2	Port Elizabeth, Uitenhage	0	1	3
Cluster 3 Ukhahlamba	**Aliwal North***, Burgersdorp Venterstad, Steynsburg	1	6	0
Cluster 4 Amatole District	East London	0	6	0

* - location of CSF infected major commercial farms

The clinical picture of the CSF infection in communal areas revealed a reported history obtained from owners, Animal Health Technicians and Veterinarians of anorexia for at least one week prior to appearance of specific CSF signs of disease and death. Some cases were reported to have died suddenly with no obvious signs while chronic cases were reported to be usually sick for an average of a month. The clinical signs of CSF observed included cachexia, pallor, weakness, a swaying gait, anorexia, dyspnea usually associated with 2˚ pneumonia, vomiting, diarrhea, seizures, skin discolouration or reddening and conjunctivitis. At necropsy the following lesions were observed:

severe swelling of tonsils, moderate to severe swelling of mesenteric, submandibular and parotid lymph nodes with peripheral haemorrhages in some. Petechial to echymotic haemorrhages of the skin, kidney, epicardium, urinary bladder and mesentery was evident. In severe cases, subcutaneous haemorrhages and oedema of the neck and thoracic area involving the lungs and heart with marked splenomegally and prominent mottling of the liver was observed. Sometimes, ascites with sero-sanguinous fluid was found in cachectic cases. Pneumonia was frequently encountered. The clinical signs of disease in commercial piggeries were similar but were more severe and also included huddling, cyanosis of the ears, snouts, feet, tails and scrotum. All pigs within an infected farm or location in a rural area including contact pigs found within a 3 km radius of the disease focus were culled. The culled pigs were placed in 4 to 6 meter deep pits excavated at suitable sites selected in conjunction with officials from water affairs. The pig carcasses were covered with lime and buried in at least 1 to 2 meters of soil. As at the end of February 2006, 225297 pigs had been culled.

A quarter of all samples tested and 48.6% of the positives originated from Amatole district municipality. The proportion of positive samples for OR Tambo and Chris Hani district municipalities were 26.2% and 12% respectively. The remaining 13% of positive samples came from the rest of the province (Table 4).

Table 4 CSF samples tested and CSF ELISA positive result distribution in the Eastern Cape Province between date and date

District Municipality	Total samples tested[1]	% of the provincial totals	CSF Elisa Positive samples	% Positive	Tissue ag pos foci[2]	Blood ag pos foci	Serologically pos foci
Ukahlamba	2483	9.71	37	2.73		13	1
Alfred Nzo	3856	15.07	66	4.87		1	1
Cacadu	4786	18.71	75	5.53	6	5	6
Chris Hani	2856	11.16	163	12.02	4	4	26
OR Tambo	5171	20.22	356	26.25	2	1	49
Amatole	6428	25.13	659	48.60	32	13	113
Combined Total	25580	100.00	1356	100.00	44	37	196

1- Total samples tested include serum, whole blood and tissues
2- Focus means a sampling point defined by a specific GPS coordinate

The presence of Classical Swine Fever Virus (CSFV) was confirmed at OVI using CSF ELISA on samples submitted from the Eastern Cape Province. Virus was detected in the tissue and blood collected from 4 of the 6 district municipalities in the province (Table 4). Only blood antigen was detected in the other 2. The number of foci positive for CSFV antibody and antigen were predominant in Amatole followed by OR Tambo and Chris Hani district municipalities respectively (Table 4).

PCR results from VLA confirmed CSFV on the 4 samples submitted from SA namely 499C/04 an isolate from Goedgenoeg, Worcester, Western Cape obtained on 13-10-04; 838C/05 an isolate from Riverside 121, Blue Crane Route, Eastern Cape taken on 22-08-05; 896C/05 an isolate from Mtlabane, Willowvale, Eastern Cape taken on 25-08-05; 915C/05 an isolate from Willowvale Village, Willowvale, Eastern Cape taken on 31-08-05. Genotyping results from VLA showed all 4 isolates to be identical and therefore from the same source of infection. The virus was found to be phylogenetically most related to 2 isolates from China (1998) and Laos (1990s) which virus has also been detected but not endemic in Europe.

DISCUSSION

The CSF outbreak between date and date in the Eastern Cape has been characterized by infection of 4 major commercial farms located in 3 different towns and by widespread infection of free roaming pigs in rural communal areas as well as pigs in townships and small holdings.

Out of the samples tested, 1224 from communal areas and 132 from commercial farms were found to be positive for CSFV. In communal areas 1083 samples were antibody positive, 1 was blood antigen positive and 140 were tissue antigen positive. The respective values for commercial farms were 65 antibody positives, 21 blood-antigen positives and 46 tissue-antigen positives. The greater number of positive samples from rural communal areas may be an indication of several factors operating. This may be a direct correlation with the higher number of samples derived from communal rural areas. However, it is also probable that the disease remained unnoticed for long in these areas compared to commercial enterprises where it is often detected quite quickly and immediate control measures are implemented. Furthermore, high Ab titres predominated in North Eastern Amatole (Mnquma/Mbashe), South Western OR Tambo (Dalindyebo /Nyandeni) South Eastern part of Chris Hani (Intsika Yethu/Ngcobo) where more than 90% of confirmed infection foci resided. All these areas consist of communal rural areas pointing to the possibility that the disease may have been present here for much longer than thought.

The detection of the infection in Centane was followed later by confirmation of CSF in a major breeding commercial sow unit in Queenstown some 200 km away. The relationship between the 2 foci of CSF infection is presently unclear. Almost all pigs in the communal rural areas are free roaming and free ranging. The spread of CSF from Centane to other communal rural areas could easily therefore be due to unrestricted movement of people, live and sick pigs and pig products from the infected zone. The fact that the owner of the breeding sow unit also owned the CSF infected farm in the Western Cape could explain how the sow unit became infected in the first place. The CSF outbreaks in the other major commercial pig farms in the Eastern Cape had a common denominator. All the commercial piggeries belonged to the owner of the infected sow unit and were all supplied weaned pigs from the same sow unit. The outbreaks in Queenstown, Cookhouse and Aliwal North commercial pig farms were also accompanied by infection in local and neighbouring townships and small holdings. However, CSF outbreaks also occurred in townships and small holdings around East London, Port Elizabeth and Uitenhage where there were no infected major commercial farms. The CSF spread within the Eastern Cape would therefore appear primarily to be due to free movement of people, pigs and pig products from infected farms and communal rural areas.

The majority of areas having many antibody positive pigs were found in Amatole district municipality followed by OR Tambo and Chris Hani districts. The same areas also had evidence of clinical disease and mortalities. ELISA antibody titres ranged from 40 to 98. Titres between 40 and 90 were detected in areas with pigs exhibiting clinical signs and mortality caused by CSF infection. Some areas showing antibody titres of over 90 did not often have any evidence of clinical disease or current mortalities. Single antigen and antibody reactors were detected in a number of areas. Single antigen or antibody positives occurring in areas where pigs displayed no clinical signs or history of disease or mortality often retested negative possibly indicating false positive reactors as this is a common problem with Flaviviruses (Darbyshire, 1960). It must however be remembered that CSFV virulence is almost always ambiguous and depends on the age, breed and immune status of the infected pigs; thus, a certain percentage of the animals which test positive without a display of clinical signs could be true positives (Mittelholzer *et al*., 2000; Floegel-Niesmann *et al*., unpublished observations). Nonetheless if any single antibody positives retested positive, the area was considered infected for purposes of this campaign and the pigs in the area were culled. In some cases mortalities were reported to have occurred one to three months previously. The latter could be attributed to the fact that CSFV infection is immunosuppressive and virus specific antibodies are slow to develop, thus making serosurveillance for the detection of new cases a futile exercise (Crauwels *et al*., 1999; Paton and Greiser-Wilke, 2003).

Phylogenetic analysis, based on the E2 gene, showed that the South African CSF virus clusters within an international group of subtype 2.1 isolates. Viruses of this genotype are prevalent in southern Asia and have been regularly implicated in outbreaks in Europe and other countries worldwide over the last 10 years. Although the SA virus is most closely related to a Chinese 2.1 isolate from 1998 and a Laotian isolate of the 1990s, other Chinese and Laotian isolates as well as other viruses from various European outbreaks cluster within this genotype. It can be concluded that the SA isolate belongs to a diverse pool of subtype 2.1 isolates that originally seem to have emerged from Asia, but that has spread to several other countries as well, including Europe (Italy, Switzerland, Austria, Germany, UK) and thus no longer can be designated solely as "Asian" strains of CSFV (VLA report).

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THE ROLE OF COMMUNICATIONS IN THE CLASSICAL SWINE FEVER ALERT IN KWAZULU-NATAL

K.D. Perrett[1]

INTRODUCTION

With the confirmation of an outbreak of Classical Swine Fever in the Eastern Cape in August 2005, the Veterinary authorities in Kwazulu-Natal (KZN) were tasked with trying to prevent the disease from entering KZN.

Classical Swine Fever (CSF) was last diagnosed in South Africa in 1918, and thus most people, both members of the public and decision makers within the department, had never heard of the disease. Communicating the presence of the disease, together with the potential consequences for the pig industry in KZN, to all role players thus became an important focus for the CSF JOCC.

MATERIALS AND METHODS

A variety of materials and methods were used to communicate with the various role-players. Most of the posters and pamphlets used were designed in-house and printed by local printing houses

Internal communications

A number of communication tools were used to try to raise awareness of Classical Swine Fever within the KZN Department of Agriculture.

Website

Following the first JOCC meeting, arrangements were made to set up a web page on the departmental website (http://agriculture.kzntl.gov.za). The web page was entitled "Classical Swine Fever Alert". The page has links to advice on biosecurity, details of the movement protocols adopted, a short summary of the disease, and gives regular updates on the disease status in KZN. At the time of writing, this page has had 1355 hits. The webpage obviously could also be accessed by people outside the department, however it was deemed to be a fast and cost effective way of distributing information within the Department.

Minutes of Meetings

Minutes of every Veterinary and JOCC CSF meeting were circulated to senior management. One of the problems of our current structure, where reporting on technical matters goes to non veterinary senior managers for action, is that the implications of a lot of the information contained in the minutes is not understood. Thus although the distribution of minutes, and thus information, within the department was timeous and accurate, it rarely elicited any response at all.

Internal Publications

Umlevo, an internal Departmental newsletter was used to highlight the role that KZN Veterinary Services was playing in keeping CSF out of the province. This publication is distributed internally to all Department of Agriculture and Environmental Affairs offices in KZN.

Posters and Pamphlets

CSF information posters in English and Zulu were displayed in the halls at the launch of the South Region at the Royal show grounds in Pietermaritzburg. Similar pamphlets were also handed out to attendees of the launch. Posters were also put up on all floors of the administration building

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at Cedara (KZN Dept of Agriculture Head Office) and pamphlets were distributed to Agricultural Extension Officers working in DC21 and DC43 – the two District Councils that border on the Eastern Cape. Pamphlets were also distributed to all SV offices throughout KZN.

External communications

These were aimed at the public in general with a specific focus being the public living close to the Eastern Cape border.

The website

While this was operational from early on in the campaign and updates were posted weekly, 1355 hits over a six month period (average = 225/month) indicates that this source of information was not widely used by the public. In addition, technical problems with the website led to significant down time which also delayed the updates being posted.

Minutes of Meetings

Minutes of all the JOCC meetings were also circulated to all role players represented on the JOCC. These included the South African National Defence Force (SANDF), South African Police Services (SAPS), the KZN Road Traffic Inspectorate (RTI), Organised Agriculture (KWANALU), Provincial Disaster Management, the South African Pig Producers Organisation (SAPPO), and the SPCA.

SAPPO and KWANALU used these minutes to keep their members informed of developments.

Government Communication and Information System

This system is made up of a network of people scattered throughout the country. In KZN the regional manager is based in Durban. The network is used to disseminate information from national government to people on the ground. They usually distribute pamphlets and posters to areas where people congregate such as clinics, shebeens, etc. We used this network to distribute 2000 posters throughout KZN. These posters, printed in Zulu, described the symptoms of CSF, outlined the various modes of transmission and appealed to members of the public to contact their nearest State Veterinarian should they have information about sick pigs.

Taxi Associations

The help of the Regional Chairman of the Taxi Associations in the South Provincial Border was enlisted to put up disease information posters in taxi ranks from Port Shepstone on the coast to Matatiele near the Lesotho border. These posters were in both English and Zulu and were aimed at the large commuter population that regularly crosses the Eastern Cape/KZN border. The posters featured a cell phone number that commuters could phone for information on the disease.

Unilever

Similarly Unilever reps were called on to assist in spreading the word by putting up the same information posters in spaza and other shops in the southern KZN region. Our request to them for assistance was handled quickly and efficiently and they even collected the posters from our office in Pietermaritzburg.

Pamphlets

Bilingual pamphlets (English and Zulu) were distributed throughout southern KZN using a variety of methods. These included:

Handing pamphlets to motorists at any one of the nine roadblocks set up along the Eastern Cape border.

House to house distribution in townships around Kokstad and Matatiele by SANDF members.

Distribution to rural communities by SANDF foot patrols

An air drop of pamphlets by the SAAF over Bizana and surrounding areas. This used English/Xhosa pamphlets sourced from the National Department of Agriculture.

Over 200 000 information pamphlets were distributed using the mechanisms described here.

Radio

The Kwazulu-Natal Radio forum consists of seven community radio stations operating in KZN as well as Ukhosi FM (a national station). The KZN Department of Agriculture has an information slot for farmers on these communal radio stations several times a week. This slot was used to disseminate information about CSF to rural communal farmers on an ongoing basis.

East Coast Radio (ECR) has comprehensive traffic reporting during the morning and evening rush hours. We used the ECR traffic updates to inform people about the presence of the roadblocks, especially over peak holiday periods when there was likely to be delays at the border for holiday makers due to the roadblocks.

Newspapers/Television

Several press releases were sent out via the Communications Section at Cedara and were printed in the local press. A TV crew visited the Eastern Cape and a short story on CSF was aired on TV2. This however was not initiated by either the KZN or Eastern Cape Departments of Agriculture.

DISCUSSION

How does one measure the success or otherwise of a campaign like this? At the time of writing – seven months after the diagnosis of CSF was confirmed in the Eastern Cape – KZN remains free of CSF. To what extent has the communication strategy contributed to this?

On a limited budget and with manpower constraints, accurately measuring the impact of the communication strategy was never a serious consideration. It could also be argued that the effectiveness of the campaign was in fact secondary to the actual conducting of the campaign. In other words the State was morally obliged to conduct the awareness campaign to the best of its' ability, regardless of how effective it was.

On the other hand, it would be advantageous for future awareness campaigns to know what worked and what didn't. This would enable more resources to be allocated, in the future, to those methods deemed to have been successful.

In an attempt to get some idea of which methods had been effective, two informal polls were conducted. The first was undertaken at the two busiest roadblocks between KZN and the Eastern Cape, while the second was undertaken at a sports day for staff held at Cedara, the KZN Department of Agriculture and Environmental Affairs headquarters.

The results were as follows:

Roadblocks

KZN resident	EC resident	Other Provinces	Heard of CSF	Not Heard	Web	Radio	Pamphlet	Poster	Other
9	27	52	77	11	0	69	34	31	27

Sports day

Heard of CSF	Not Heard	Web	Radio	Pamphlet	Poster	Other
81	6	11	38	14	10	32

The “other” column was made up mostly of word of mouth and newspaper articles. Some participants had heard of CSF through more than one channel.

CONCLUSION

While this small survey cannot claim to be statistically significant for the population of KZN, it does provide an indicator for where more emphasis might be placed in future disease awareness communication efforts.

GENETIC DIVERSITY OF *EHRLICHIA RUMINANTIUM* IN SOUTH AFRICA

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SUMMARY

Heartwater is caused by the tick borne pathogen *Ehrlichia ruminantium*. It is a disease that occurs in cattle, sheep and goats and in wild ruminants. The currently available blood vaccine is expensive to produce, requires an uninterrupted cold chain for delivery and provides only limited protection against heartwater. The vaccine is therefore not suitable for use in rural areas. Current investigations, by researchers at ARC-OVI, in vaccine development have indicated that considerable genetic diversity exists among isolates in the field. This explains the limited protection obtained with the current blood vaccine. These investigations have highlighted the importance of detailed knowledge of isolates present in the field for vaccine development. However, scant epidemiological information is available from areas particularly badly affected by the presence of heartwater in both the commercial and communal farming sectors. Therefore an in-depth epidemiological study is currently ongoing in heartwater endemic regions of South Africa. The specific and sensitive pCS20 diagnostic test was used to detect *E. ruminantium* in ticks and blood of domestic ruminants and sequencing of the corresponding pCS20 DNA fragment was used to distinguish between and to identify new isolates. Only 24 % of samples collected from heartwater endemic areas in South Africa tested positive for *E. ruminantium* and seven new isolates were identified. Two of these isolates were established in culture and a cross protection study indicated that they do not protect against the highly virulent Welgevonden isolate but that the Welgevonden isolate protects against them.

INTRODUCTION

Heartwater is an economically important disease for the red meat and wool industries in SA and it is caused by the tick borne pathogen *Ehrlichia ruminantium* (occurs in cattle, sheep and goats and in wild ruminants). The disease is widespread in Sub-Saharan Africa and Madagascar and it is also present on 3 neighboring islands in the Caribbean (Uilienberg G. 1996). Heartwater is transmitted by ticks of the genus *Amblyomma* of which *A. hebraeum* is the only known vector in South Africa (Bezuidenhout *et al*. 1987).

Currently, immunisation of animals relies on the infection and treatment method where animals are infected with blood containing the Ball3 isolate and treated with tetracycline once a febrile response is detected. This vaccine is expensive to produce, requires an uninterrupted cold chain for delivery and is therefore not suitable for use in rural areas. Because it contains live *E. ruminantium* organisms, it cannot be used outside S. African endemic areas. Furthermore, there is considerable genetic diversity among isolates in the field, which may explain the limited protection against heartwater obtained with the vaccine. Because of this many farmers practice dipping to reduce tick infestation.

The specific and sensitive pCS20 diagnostic test is used to detect *E. ruminantium* in ticks and blood of domestic ruminants (Van Heerden *et al*, in Press). Sequencing of the corresponding pCS20 DNA fragment is used to distinguish between and to identify new isolates. There is also a recombinant ELISA (the MAP1B) which detects animals which have had recent exposure to *E. ruminantium* but neither test will detect otherwise healthy carrier animals with 100 % certainty (Peter, T.F., and Jongejan, F. 1998).

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Since only scant epidemiological information is available from areas particularly To establish new E. ruminantium cell-lines, the BA 886 (Yunker *et al*, 1988) cell line is used as host cells for *E. ruminantium*, and is propagated in DF-12 medium. Cells are cultured as monolayers at 37 °C in a humidified atmosphere of 5 CO_2 in air.badly affected by the presence of heartwater in both the commercial and communal farming sectors an in-depth epidemiological study is being undertaken in Heartwater endemic regions of South Africa. In this study 24 % of samples tested positive for *E. ruminantium* and seven new isolates were identified (by sequencing). Two of these isolates were established in culture. To establish new E. ruminantium isolate in culture the BA 886 (Yunker *et al*, 1988) cell line is used as host cells for *E. ruminantium*, and is propagated in DF-12 medium. A cross protection study indicated that only one new isolate protects against the highly virulent Welgevonden isolate but that the Welgevonden isolate protects against both. A geological information system was used to plot the new isolates on a map.

MATERIALS AND METHODS

Collection of samples

Amblyomma hebraeum ticks and blood samples from cattle, sheep and goats were collected from designated farms in heartwater-endemic regions of South Africa. These regions include the Eastern Cape, Kwazulu Natal, Mpumalanga, Limpopo, Gauteng and North West Provinces. Stabilates were prepared at a ratio of 1:1 using sucrose potassium glutamate (SPG) and blood. Live ticks were homogenised in 1.35 µl SPG.

Identification and characterization of *E. ruminantium* in samples

The pCS20 PCR diagnostic test (specific for *E. ruminantium*) was used to determine the presence of *E. ruminantium* in the blood and ticks. PCRs were performed with 5 µl of either blood or tick sample as template in a 50 µl reaction with the primers HH1 (5'-CCC TAT GAT ACA GAA GGT AAC CTC GC-3') and HH2 (5'-GAT AAG GAG ATA ACG TTT GTT TGG-3'). The amplicon is ~900-bp within the 1,306-bp pCS20 sequence of *E. ruminantium*. The pCS20 DNA fragment of samples that tested positive with the pCS20 PCR was sequenced (ABI Prism 3100 Genetic Analizer Applied Biosystems. The TaKaRa EX Taq (TAKARA SHUZO CO., LTD Japan) enzyme was used. PCR conditions were: Incubation of 25 seconds at 94 °C, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 62 ° for 45 sec, elongation at 72 °C for 30 sec, final elongation at 72 °C and hold at 4 °C (Gene Amp PCR System 9700, Applied Biosystems). Each set of PCRs included a positive control of 1 µl purified genomic DNA *of E. ruminantium* (Welgevonden) and a negative control containing 5 µl distilled H_2O (Van Heerden *et al*, in Press). The pCS20 positive samples were further characterised by PCR amplification and sequencing of the 16S gene.)

Establish new genotypes in culture

Infection of cell BA 886 monolayers

A merino sheep was infected with *E. ruminantium* by intravenous injection of 10 ml blood stabilate from a sick sheep from Springbokfontein. The Body temperature of the sheep was monitored daily and a blood sample was drawn, from the jugular vein into sterile Vac-u-test^R tubes containing heparin (lithium heparin, 14.3 USP/ml blood) as anticoagulant on the third day of febrile reaction when it rose to 42° C. The infective blood (3 ml) was inoculated into each of several 25 cm² culture flasks containing a confluent layer of BA 886 cells. The cultures were incubated at 37° C for 2 to 3 h on a rocking platform at 3 cycles/min after which the infective inoculum was decanted and the cell monolayer was rinsed with 3 x 5 ml of PBS. Five ml DF-12 medium was added and incubation was continued.

Heavily infected cultures were harvested for sub cultivation by scraping off the cell monolayer into the medium using cell scrapers. Cells were dispersed by pipetting the suspension up and down with a variable pipette (0.5 to 5 ml, Socorex, Lausanne, Switzerland) and the suspension was then transferred

at a ratio of 1:2 or higher on to new endothelial cell monolayers. After 24 h the entire medium was discarded and replaced with 5 ml fresh DF-12 medium.

Microscopic examinations were carried out after staining as follows in order to determine the presence of *E. ruminantium* in the endothelial cells. Small samples were removed from the monolayer using a sterile 21 gauge needle with a bent tip and smears were prepared which were air-dried, methanol-fixed and stained with RapiDiff (Clinical Sciences Diagnostics, Booysens, South Africa).

Determine cross-protection status of new isolates relative to other isolates

The new cultured isolates were titred in Heartwater free sheep. Cross-protection studies were done using the new genotypes, and the Welgevonden isolate (currently shows widest protection range). The immunised sheep received a heterologous challenge two months later and were monitored for signs of disease and a reaction index was determined (Collins NE *et al.* 2003). The sheep that were used for this study were monitored for temperature reaction every day and sick animals were treated with tetracycline.

RESULTS

A total of 574 samples of blood and ticks were probed with pCS20. Of these only 47 blood samples and 92 tick samples probed positive with the pCS20 probe. Nineteen of the pCS20 positive samples were further characterised by sequencing the corresponding pCS20 fragment. Seven new isolates were identified and their locatia indicated in fig.1. The pCS20 sequence of five samples from different farms was identical to that of the Rockhurst isolate. (Table 1) and 1 of each of the following isolates: Silverdale, Argyll, Glendowan1, Glendowan2, Springbokfontein1 (SBF) and Springbokfontein2. Eight samples were also characterised by sequencing the 16S gene (Table 1). Interestingly, 2 of the samples with Rockhurst pCS20 sequence had different 16S genes (Table 1). Comparison of the Rockhurst isolate with the Blaauwkrans, Mara87/7 and Welgevonden isolates show diversity in the sequence (from basepare (bp) 685). The new isolate and the existing Blaauwkrans isolate sequence was identical from the start of the sequence to base pair 684 and from base pair 685 to the new Rockhurst isolate and Mara87/7 was identical (Table 2). This suggests that Rockhurst could have resulted from a recombination of Blaauwkrans and Mara87/7 isolates (Table 2).

The two SBF isolates were successfully cultured and tested in a cross protection study. Neither of these is more virulent than the Welgevonden isolate although SBF2 does cross-protect against Welgevonden challenge.

In conclusion, of the 19 samples of blood and ticks, which were further characterised by pCS20 sequencing, five were new isolates. These isolates were named after the farm where they were first isolated. The new Rockhurst isolate was identified on five different farms. The following isolates was discovered: Silverdale, Argyll, Glendowan1, Glendowan2, Springbokfontein1 (SBF1) and Springbokfontein2 (SBF2).

The 16S gene was sequenced from 9 samples. These samples were identical to either the Welgevonden, Mara87/7 or Omatjenne isolates.

Table 1. Characterisation of isolates occurring in ticks and blood collected from farms in heartwater endemic areas in South Africa.

Province	Town	Farm	Sample	pCS20	16S
				Sequence#	Sequence#
MP	Eglington	Dip tank	Tick	Mara87/7#	ND
EC	Grahamstown	Rockhurst	Blood	Rockhurst*	Welgevonden
EC	Grahamstown	Henley	Tics	Rockhurs*t	ND
EC	Grahamstown	Riverside	Tick	Rockhurst*	ND
EC	Grahamstown	Riverside	Blood	ND	Omatjenne
EC	Grahamstown	Henley	Tick	Mara87/7#	ND
EC	Grahamstown	Glendowan	Tick	Glendowan1*	ND
EC	Grahamstown	Glendowan	Tick	Glendowan2*	ND
EC	Grahamstown	Glendowan	Tick	Mara87/7#	ND
EC	East London	Little Chenunga	Blood	Rockhurst*	Mara87/7
EC	East London	Sunny Side	Blood	ND	ND
EC	East London	Fountain Spr	Blood	Mara87/7#	ND
EC	East London	Silverdale*	Tick	Silverdale*	ND
EC	East London	Blow Ridge	Tick	Blaauwkrans#	ND
EC	East London	Blow Ridge	Tick	Rockhurst*	ND
KZN	Msinga	Dip tank	Blood	ND	Omatjenne
KZN	Amandlalathi	Dip tank	Tick	Mara87/7#	Mara87/7
KZN	Port Shepstone		Tick	Mara87/7#	Mara87/7
KZN	Vryheid	Argyll	Blood	Argyll*	ND
GAUT	Pretoria	Roodeplaat	Blood	Kumm2#	ND
LIMP	Potgietersrus	SBF	Blood	SBF1*	Welgevonden
LIMP	Potgierersrus	SBF	Blood	SBF2*	Welgevonden

* - First isolated on this farm; ND – not done; # - sequence identical to stock X
SBF –Springbokfontein

Table 2. Identification of the diversity between the New Rockhurst isolate with exiting isolates

	bp 191	bp 210	bp 243	bp 320	bp 380	bp 616	bp 685	bp 694	bp 704
Blaauwkrans	G	A	C	C	G	T	T	C	G
Rockhurst	G	A	C	C	G	T	C	T	A
Mara87/7	G	A	C	T	G	C	C	T	A
Welgevonden	A	C	T	T	A	C	T	C	G
	B	B	B	B	B	B	M	M	M

bp – base pair B – Blaauwkrans isolate M – Mara87/7 isolate

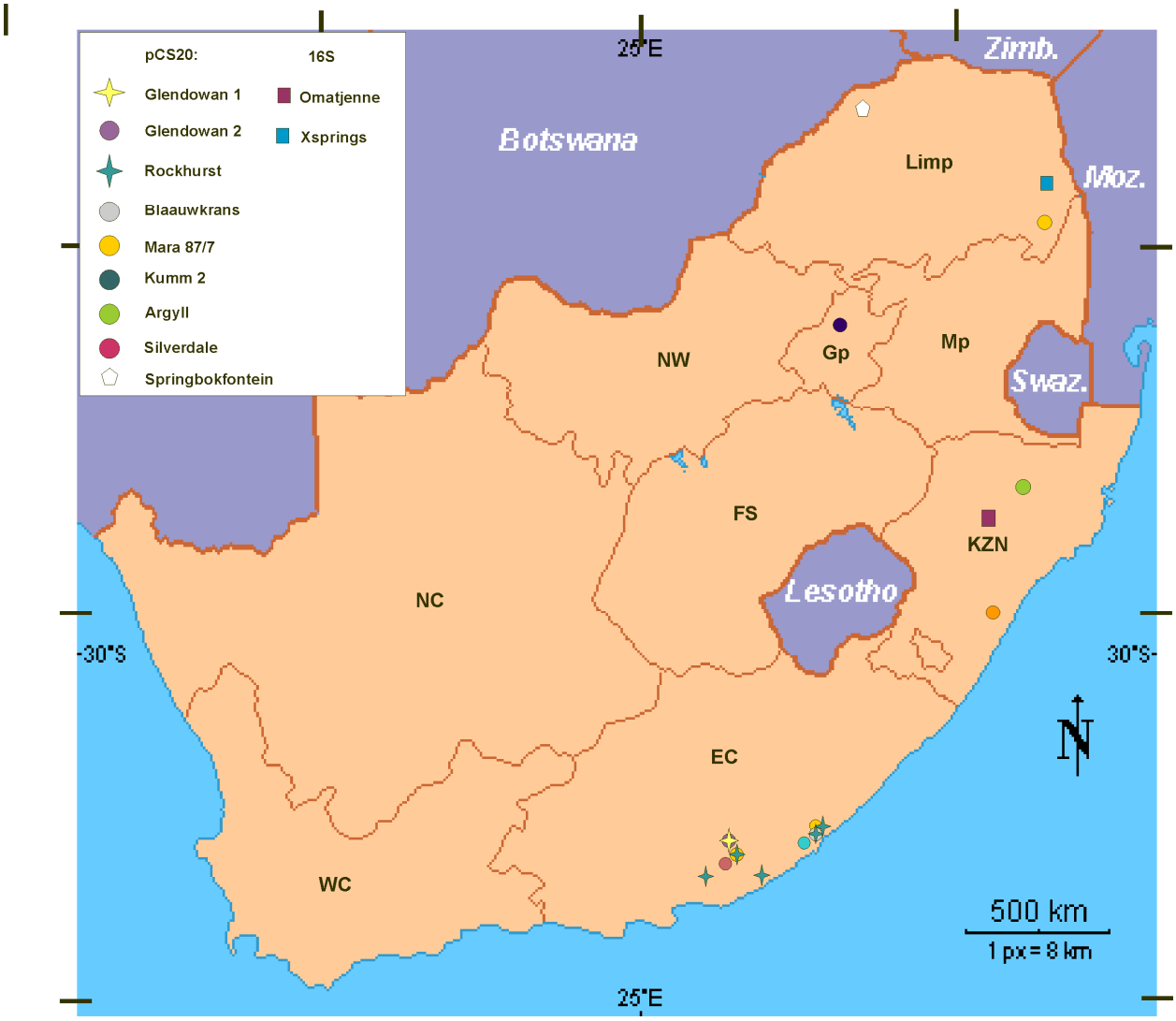

Figure 1. Geological distribution of Heartwater

DISCUSSION

The identification of seven new isolates the Rockhurst, Silverdale, Argyll, Glendowan1, Glendowan2, Springbokfontein1 (SBF1) and Springbokfontein2 (SBF2) shows that genetic diversity of *E. ruminantium* isolates do exist in the field.

The cross-protection study undertaken between the well characterised Welgevonden stock and the two new SBF1 & SBF2 isolates indicated that only the SBF2 isolate cross protected. SBF2 is the first isolate that has been found to protect animals against a challenge with the Welgevonden stock. Unfortunately a similar study was not undertaken with the vaccine Ball3 stock. However, we do know that animals that are immune to the Ball3 stock (vaccine stock) succumb to heartwater when challenged with the Welgevonden stock (Collins NE *et al.* 2003). Therefore it will be highly likely that animals immune to Ball3 stock would similarly succumb to heartwater when challenged with the SBF2 isolate.

The data presented here highlight the genetic diversity of isolates and explains why only limited protection is obtained with the blood Ball3 vaccine in the field. Continual characterisation of new isolates will benefit the development of an efficient vaccine against heartwater.

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A STOCHASTIC MODEL FOR DETERMINING EXPOSURE DOSES FOR BEEF CATTLE USED FOR *IN SITU* MONITORING OF COMPLEX METAL EXPOSURES WITHIN THE VANADIUM INDUSTRY

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SUMMARY

A sentinel herd of 30 Brahman Cross cattle was run as an extensive beef commercial herd and experimental cohort on a vanadium mining property over a five year period. The cattle were farmed as two groups. A high exposure group that grazed downwind in an area immediately adjacent to the processing plant and a low exposure group whose grazing began approximately 2 km upwind of the processing plant. A primary objective of the study was to investigate whether a long-term animal sentinel system for the vanadium mining industry could act as a monitoring system for pollution problems and thus address the concerns of surrounding farmers. Various potential biomarkers and production and health parameters were monitored in the cattle over the five-year period. However, before the consequences of exposure and the value of the biomarkers could be assessed it was necessary to produce a method for quantifying how much vanadium the cattle had been taking in over the duration of the trial and what forms of vanadium were playing a role. The method had to account for chronic field exposures where there is a fluctuation of exposure over time and account for individual animal variability.

A systems approach was used to assess the risks related to the cattle in the vicinity of the mine. This approach offered a structured framework for dealing with the five years of accumulated data. This paper describes, within this framework, a qualitative method for hazard identification and release assessment within the vanadium mining industry and a quantitative stochastic model for assessing persistent long-term environmental exposure to vanadium. The stochastic nature of the model deals with uncertainty and variability within the available data. The primary inputs were direct measurements at the point of exposure and account for oral (grass, soil and water) and inhalation exposure (aerial fall out). This distinguishes it from Gaussian plume dispersion models. The primary inputs are combined with the physiological parameters of the species concerned to provide an accurate estimate of the dose of vanadium intake. The final output of the model is a distribution curve of probable vanadium intake values based on the variability within the inputs. The exposure model estimated an external dose over the five-year period of between 0.07 and 25 mg vanadium/kg body weight/day (0=2.8) for the High Exposure group and between 0.05 and 14 mg/kg/d (0=1.5) for the Low Exposure group. There was only a 5% probability of values being <0.5 or >8.17 mg/kg/d for the High Exposure group and <0.4 or >3.44 mg/kg/d for the Low Exposure group.

INTRODUCTION

Based on experience with an outbreak of vanadium poisoning in 1991 (Gummow *et al.*, 1994), the authors were approached in 1999 by another vanadium mining company, which had been having complaints by surrounding farmers that cattle were being affected by their mining process. The mine wished to know if there was a scientific basis for determining whether these allegations were founded or not. Because there was insufficient information published about the effects of chronic low-grade exposure to vanadium, the question could not be answered. It was therefore

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suggested that a sentinel herd of cattle be run on the mine that could be monitored by an independent body, such as the Veterinary Faculty at the University of Pretoria.

The concept of a sentinel herd was expanded into an experimental design to enable biomarkers for vanadium exposure in cattle to be assessed. Biomarkers were of interest because urine, which is used in occupational health monitoring, is not a very reliable indicator of exposure. Thus alternative ways of establishing exposure would help the industry. At the same time the effects of chronic low-grade vanadium exposure could be assessed, thus addressing the concerns of the surrounding communal farmers. Coupled to this was the question as to whether the meat and milk was safe for human consumption

Another objective of the project was simply to see if it was practical for a mine to manage and run a herd of cattle and whether this could be used as a risk communication tool with respect to the media and farmers.

MATERIALS AND METHODS

The design comprised a cohort of 30 cattle divided into a low exposure group of 20 cattle and a high exposure group of 10 cattle. The low exposure and high exposure areas were selected on known background concentrations of vanadium in the soil and proximity to the processing plant. The cattle were farmed extensively to ensure maximum oral exposure through grazing and the number of cattle was determined by the available grazing on the 200 ha of available land. The project was managed and funded by the mine and subsidized to a limited extent by the Faculty of Veterinary Science, University of Pretoria and the Institute of Soil, Climate and Water of the Agricultural Research Council. The herd was run on a commercial herd basis and was visited every 3 months during which time a variety of samples were taken. These included bone, faeces, urine, hair, milk and blood. Data was collected for a period of five years.

It was decided that many of the objectives could be met by following a risk analysis process similar to that recommended by the OIE for import of animals and animal products. This involved evaluating the data accumulated over the five years in a systematic fashion, beginning with a Hazard Identification, followed by a Risk Assessment, which leads into Risk Management and at all stages involves Risk Communication. The Risk Assessment portion of the system is further sub-divided into Release Assessment, Exposure Assessment, Consequence Assessment and Risk Estimation. Once a risk estimation is available it is possible to do a Risk Evaluation, work out what options are available, implement them and continue monitoring the process.

Because of the magnitude of the project, this paper only focus's on the Hazard Identification process, the Release Assessment and most importantly the Exposure Assessment.

Hazard Identification was done using a qualitative process based on examination of the literature with respect to vanadium mining, previous case histories and preliminary findings of tissue concentrations of metals in cattle in the trial. It also involved interviews with mine management.

The purpose of the Release Assessment was to establish where the potential hazards could be derived from and how they are likely to be released. This was established in two ways, firstly by going through the various steps required in the manufacture of vanadium pentoxide, the end product of the mine, and secondly by using a qualitative method to weight the significance of each release point. This process involved a number of interviews with experts in the mine.

The Exposure Assessment was based on measurements taken at various sampling points within the high exposure camps and the low exposure camps as illustrated in Fig. 1. Grass and soil samples were taken on a quarterly basis over the five year period and aerial fall out samples were collected in buckets of distilled water and analysed on a mean monthly basis. The advantage of the method is that it is a relatively simple technique to carry out and measurements are direct measurements at the point of vanadium intake or exposure and not extrapolated measurements as would be the case with for example plume dispersion modeling. It is assumed that cattle move and graze evenly throughout the area of exposure during each measurement period.

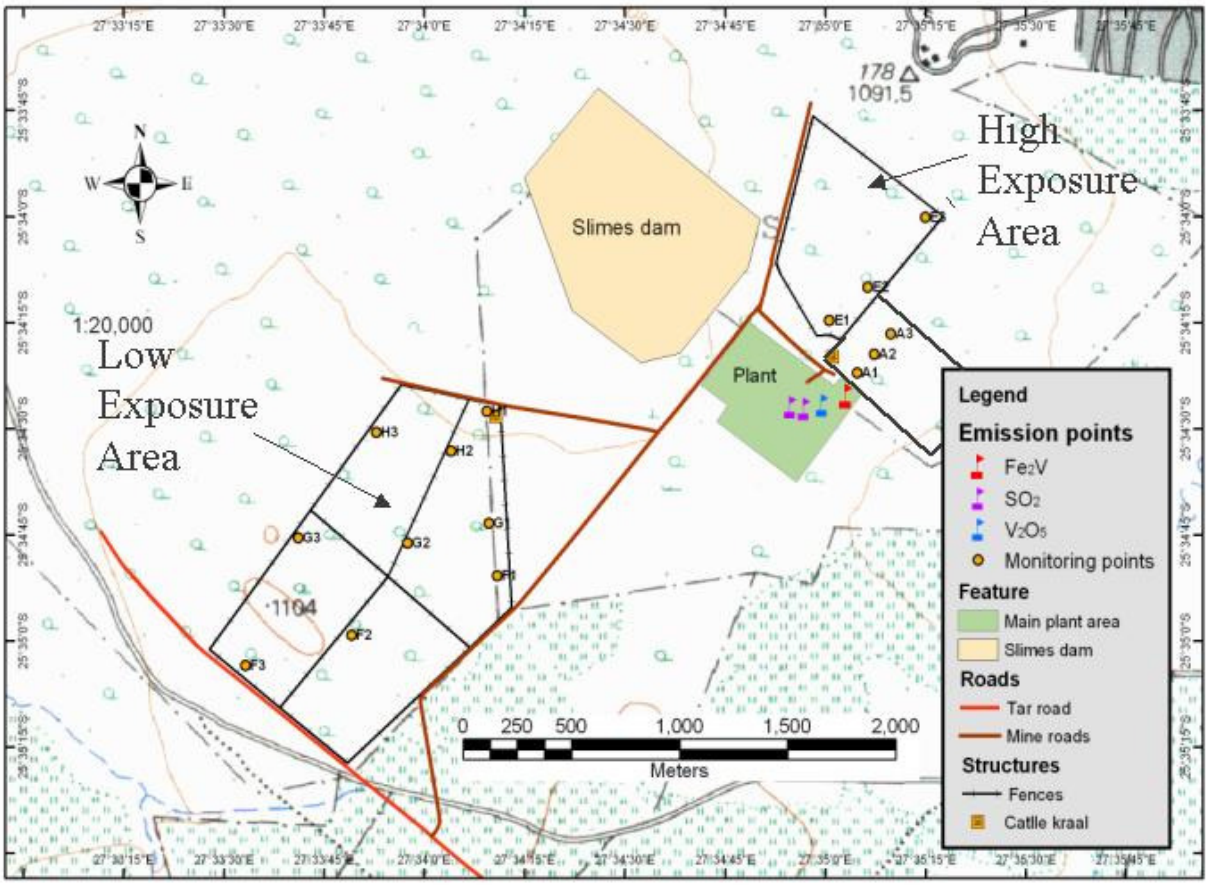

Figure 1. Map of the area where the trial took place showing the primary sources of vanadium release in relation to the low exposure and high exposure camps and the points where samples were routinely collected in those camps.

The objective of the Exposure Assessment model was to simulate the amount of vanadium that cattle would have taken in during the project. The model uses a stochastic simulation process that incorporated distribution functions defined by the input data and an iteration process called Latin-Hypercube sampling. The engine of the model is a Microsoft Excel add-in programme called @Risk, which is sold by a company called Palisade Corporation in the USA. The data comprised the aerial fall out data, the unwashed grass concentrations of vanadium, the EDTA soluble soil concentrations of vanadium, and background water concentrations of vanadium over the duration of the trial. The data from each of these four inputs was described separately by means of a truncated lognormal distribution function. This function was generated by a data-fitting programme called Bestfit, which is also part of the Palisade Corporations set of add-ins. The lognormal distribution function was chosen because it was consistently within the top ranking distribution functions that Bestfit fitted to the data and because it is commonly used for environmental data.

RESULTS AND DISCUSSION

The primary hazardous substance was obviously vanadium, but the Hazard Identification process went further in being able to identify the various forms of vanadium that constituted hazards. Other potential hazards included sulphur compounds and ammonium gasses, released during the manufacturing process. The screening of animal tissues and soil and grass for other metals and minerals failed to show any other elements that were abnormally high.

Interviews with mine management about the Release Assessment was found to be an important exercise in the context of the whole project as it forced the animal scientists to sit down and understand the chemistry of the process and it forced mine management to take cognisance of the potential adverse effects of that process. Until this was done, both sides failed to really understand the link between mining activities and farming process. The result was two tables. Table 1 gives broad overview of potential sources from which compounds could be released and the weight that each plays in the release of a specific compound. Table 2 looks more specifically at stack emissions in greater detail. High indicates that that stack contributes highly to the emission of that particular compound and would therefore be a primary source. No implies there is no output of that particular compound from that source. This process therefore helps to localise the most important release sites with respect to specific compounds. The importance of this comes back into play when doing the consequence assessment.

Table 1. Sources of vanadium and the weight of their contribution to the release of vanadium into the environment

Source	**Total V (%)**	**V_2O_5**	**$NaVO_3$**	**NH_4VO_3**	**V_2O_3**	**SO_2 (%)**
Stack Emissions	66	High	Low	Medium	No	50
Calcine Dumps	23	V Low	High	No	No	0
Natural and Historical Background	0.5	No	No	No	No	0
Roads	9.5	No	No	No	No	0
Underground Seepage	3	No	Low	No	No	0
Scrubber Pond	0	No	No	No	No	50

Table 2. Sources of stack emissions and the degree to which each compound is released from that source

Stack Sources	**V_2O_5**	**$NaVO_3$**	**NH_4VO_3**	**V_2O_3**	**SO_2**	**H_2SO_4**	**NH_3**	**PM10**	**TSP**
1.Old deammoniator stack	High	Low	Medium	No	Low	No	High	Low	Low
2.New deammoniator stack	High	Low	Medium	No	Low	No	High	Low	Low
3. Kiln main stack	No	V Low	No	No	High	Low	Low	High	High
4. FeV stack	Low	No	No	No	No	No	No	Low	Low
5. Slag Bag house stack	Low	No	No	No	No	No	No	Medium	Medium
6.Dispatch bag house stack	High	No	No	No	No	No	No	Low	Low
7. Furnace stack	High	No	No	No	V Low	No	No	Low	Low
8. Desilication stack	Low	V Low	No	No	No	Low	No	No	No
9. Raw materials bag house stack	Medium	No	No	No	No	No	No	Medium	Medium
10. Boiler stack 1	Low	No	No	No	High	No	No	High	High
11. Boiler stack 2	Low	No	No	No	High	No	No	High	High

PM10 = particulate matter with an aerodynamic diameter of <10 Φm; TSP = total suspended particles

The environmental inputs for the Exposure Assessment model are shown in Fig. 2, 3 and 4. They reflect the grass, soil and aerial fall out vanadium concentrations that occurred over the five-year period. The information gathered has been defined as a mathematical function which the computer uses to generate potential values that could occur and the probability of them occurring. When the model runs, what the computer is doing each time an iteration is run is sample a value, without replacement out of these curves. These values are then used to generate the final output. This is done simultaneously for soil, grass, fall out and water vanadium concentrations.

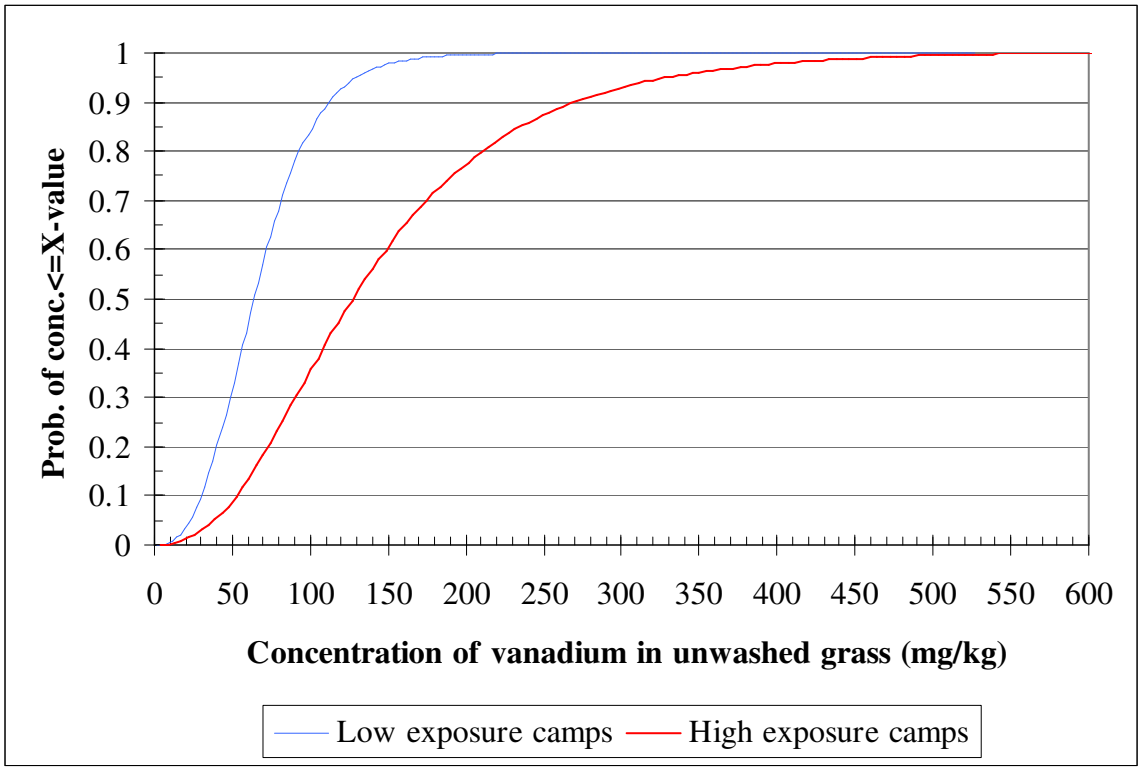

Figure 2. Distribution curves for the concentration of vanadium in unwashed grass over five years

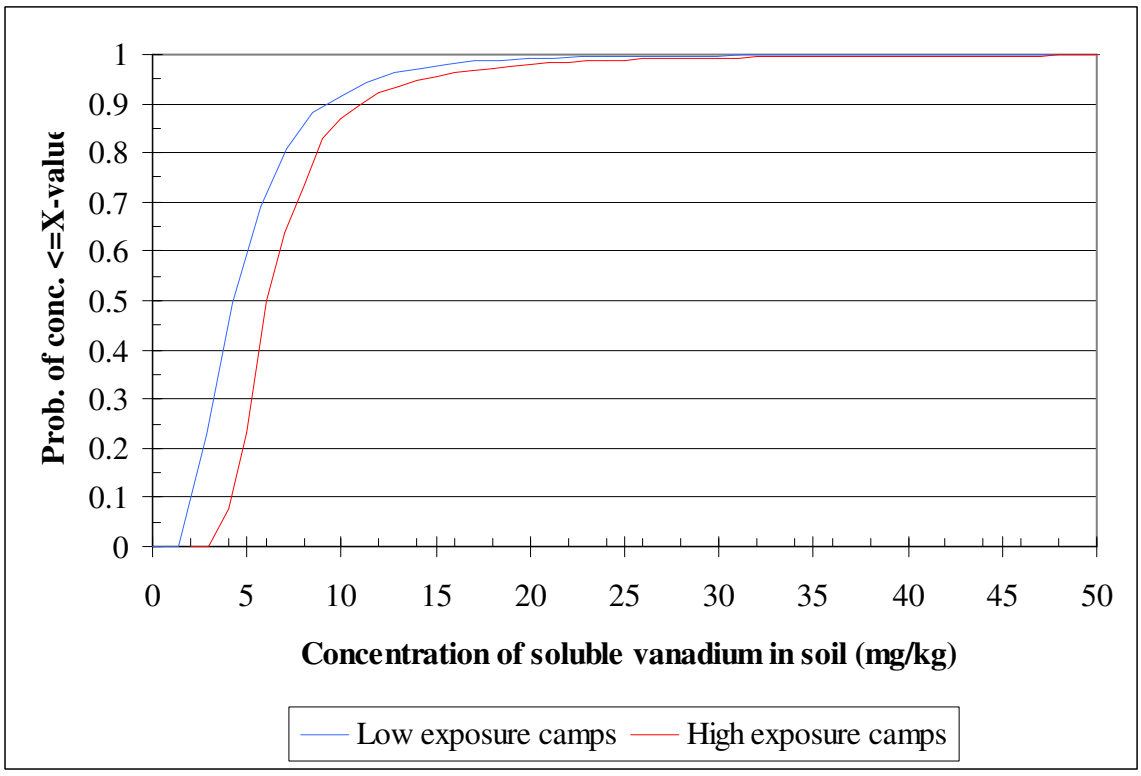

Figure. 3. Distribution curves of the concentration of soluble vanadium in soil over the five-year period

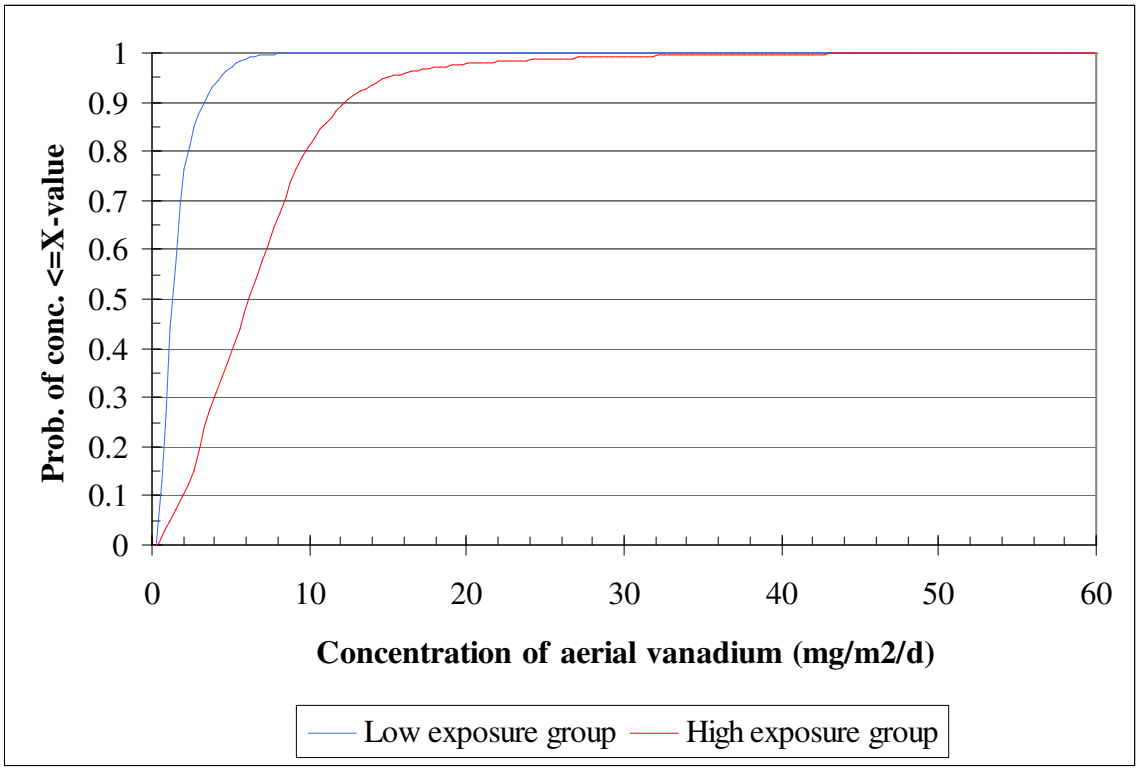

Figure. 4. Distribution curves for the concentration of aerial fall out vanadium over five years ($\Phi g/m^2/d$)

Those functions that define the raw data are then slotted into the physiological portion of the model as follows: Beginning with grass, the intake of grass by the cattle is calculated as dry matter intake (DMI) which is a function of the body mass. The body mass for each group was defined as a normal distribution function based on the weight measurements of cattle in each group over the period of the trial and the DMI is based on a percentage of that weight. Based on these inputs the computer generates possible values for the amount of grass ingested, which is coupled to the amount of vanadium occurring in the grass to give how much vanadium the cattle in the respective camps was ingesting by eating grass. (Table 3)

The same approach is used to calculate the amount of vanadium ingested in the water and the amount of vanadium ingested by cattle through eating soil. By adding up the grass, water and soil intake it is possible to get a set of values for the total amount of vanadium ingested per cow per day. As can be seen in Table 3, the high exposure animals were ingesting roughly twice as much as the low exposure animals.

Table 3. Exposure Assessment model showing inputs, distribution functions and outputs

	HIGH	LOW	Comment
Oral Intake Per Day			
Grass (veld) diet			
Adult Cows (>24 m)	465	437	kg
Body Weight (stdev)	74	67	From trial data
Body Weight (mean)	530	495	From trial data
Body Weight function	522	493	RiskNormal,Risk truncate(250,650)
% of Body Weight	0.0175	1-2.5%	(RiskUniform(0.01,0.025))
Mass of Grass Ingested per day - DMI	9.1	8.6	kg
Vanadium in grass (mg/kg)	165	77	From unwashed grass model
Vanadium ingested in grass	1507	664	mg/d

Water			
Volume of water ingested (Cows (>24 m)) (l)	38.73	36.62	WI=0.075+4.234*DMI+-1.15
Vanadium in water)	0.024	0.0245	Φg/l
Vanadium ingested in water	0.93	0.9	Φg/d
Vanadium ingested in water	0.00093	0.0009	mg/d
Soil Ingested			
% of DMI	0.095	1-18% DMI	RiskUniform(0.01,0.18)
Amount of soil ingested	0.88	0.82	kg
Soluble Vanadium in soil (mg/kg)	6	5	From EDTA soil model
Vanadium ingested with soil intake	5.2	4.1	mg/d
Total vanadium ingested per cow per day	1512	669	mg/d
Inhalation			
Vanadium concentration in air (Φg/m^2/d	8882	15006	From aerial fall-out model
Ground air speed mean * prev wind dir	3.87	3.13	From weather station data
Ground air speed stdev * prev wind dir	2.08	1.78	
RiskNormal ground air speed * prev wind dir	4.02	3.29	km/h
Wind speed * prev wind dir	1.12	0.91	m/s
Diameter of collector	0.185		m
Radius of collector	0.0925		m
Gravitational acceleration constant [g]	9.8		m/s2
Pi constant	3.14		
Terminal Velocity inputs			
Radius if V particles [r] (m)	0.00005		RiskUniform($4x10^{-6}$,$6x10^{-6}$)
Density of V2O5 [p]	3357	kg/m3	g/ml
Density of the air [σ]	1.2		g/l @ 25C = kg/m3
Viscosity of the air [η]	0.000018		Pascal sec
Vertical terminal velocity (v_t)	1.01		m/s
Time for particle to move over collector (Δt)	0.17	0.2	s
Change in vertical distance in time (s_{vert})	0.18	0.21	m
Volume sampled every Δt seconds	0.0045	0.0055	m3
Volume sampled in 24 hrs	2357	2357	m3
V conc. in m^3 air	3.77	0.64	ug/m3/d
V conc. in litre air	0.0038	0.00064	ug/l
Respiratory rate	30		RiskNormal(30,1.8,RiskTruncate(15, 60))
Tidal volume	3.5		RiskNormal(3.5, 0.4)
Respiratory rate per day (24 h)	43200		
Respiratory volume= Resp freq x tidal volume	1296000		Litre per day
V inhaled per day	4883	828	Φg/d
Amount of V inhaled per day	4.88	0.83	mg/d
Exposure dose of vanadium per day	1517	670	mg/d
Exposure dose of vanadium per kg per day	2.86	1.35	mg/kg bwt per d

Distribution Function Mean	**2.78**	**1.46**	**OUTPUT(mg/kg/d)**
Standard Deviation	2.64	1.04	
Min	0.07	0.05	
Max	24.58	13.81	
5 Percentile	0.49	0.36	
50 Percentile	1.89	1.2	
95 Percentile	8.17	3.44	

The inhalation portion of the model was more complex because fall out of vanadium was measured in ug/m^2/d. This therefore had to be converted into the concentration of vanadium per litre of air to enable the amount of vanadium inhaled to be calculated. The mean ground air speed over each relevant time period was calculated using data captured by the weather station set up on the mine for this purpose. The wind speeds were stratified according to wind direction, and the mean wind speed calculated for winds blowing in the direction of the high exposure and low exposure camps. To take into account the difference in predominant wind direction, the mean wind speeds were weighted by the frequency that the wind blew in the direction of the high exposure and low exposure camps respectively.

To estimate the amount of vanadium inhaled, the vertical terminal velocity of a V_2O_5 particle was calculated according to the formula:

$$v_t = \frac{2r_v^{\,2}(\rho - \sigma)g}{9\eta}$$

where r_v = radius of vanadium particle; ρ = density of V_2O_5; σ = density of air; g = gravitational acceleration and η = viscosity of air (Whelan & Hodgson, 1979). Inputs for the formula were obtained from the CRC Handbook of Chemistry and Physics (1977), with the exception of the radius of vanadium particles, which was derived from expert opinion obtained from the mines engineers. The radius was therefore modelled as a Uniform distribution function representing the range 30 to 60 Φm.

The time for particles to move horizontally over the fall out bucket (Δt) was calculated by dividing the diameter of the fall out bucket (N) by the weighted wind speed (ws). To determine the height of the column of air that contributed to the vanadium deposited in the bucket, this time was then multiplied by the vertical terminal velocity (v_t) to give the change in vertical distance of particles in this time (Δs_{vert}). The volume of air sampled in time delta t was then calculated using the formula:

$$V_{air} = \Pi r^2 \times \Delta s_{vert} = \frac{\Pi r^2 \times v_t \times \phi}{ws}$$

where r is the radius of the fall out buckets. The volume of air sampled in a 24 h period could then be calculated as: $\frac{V_{air}}{\Delta t} \times 24h \times 3600\,s = \Pi r^2 \times v_t \times 24\,h \times 3600\,s$. Hence the volume of air sampled in 24 hours is directly proportional to the fall out surface area and vertical terminal velocity of the vanadium particles.

The fall out concentration of vanadium was then divided by the volume of air (V_{air}) to give the concentration of vanadium per m^3 of air. This was converted to vanadium concentration per litre of air by dividing by 1000. The respiratory rate per minute of an adult bovine was modelled using a Normal distribution function with a mean of 30 and standard deviation of 1.8, truncated at 15 and 60 (Svendsen & Carter, 1984; Reece, 1991). This was multiplied by 60 x 24 to give the 24 h respiratory rate. The tidal volume was modelled using a Normal distribution function with a mean of 3.5 and a standard deviation of 0.4 (Svendsen & Carter, 1984). The daily respiratory volume could then be calculated by multiplying the tidal volume by the 24 h respiratory rate. This was then multiplied by the vanadium concentration per litre of air to give an estimation of the amount of vanadium inhaled by an adult bovine per day.

The output of the model was then the sum of the oral daily dose of vanadium and the daily inhalation dose of vanadium to give a distribution for the daily exposure dose for cattle in the high exposure camp and low exposure camp (Fig. 5).

What does the output of the model tell us? During the 10000 iterations that the model is run the computer is randomly sampling values from within each of the defined input distribution functions and ends up giving a distribution curve of possible values that could occur given the variability and uncertainty in the inputs and the probability of those values actually occurring. So for example, it can be seen from the output (Fig. 5) that we can be 95% certain that cattle in the high exposure camp received less than 8.17 mg/kg vanadium per day and 3.44 mg/kg in the case of the low exposure camp. On average they are likely to have received 2.78 mg/kg/d in the high exposure group and 1.46 mg/kg/d in the low exposure group (Table 3).

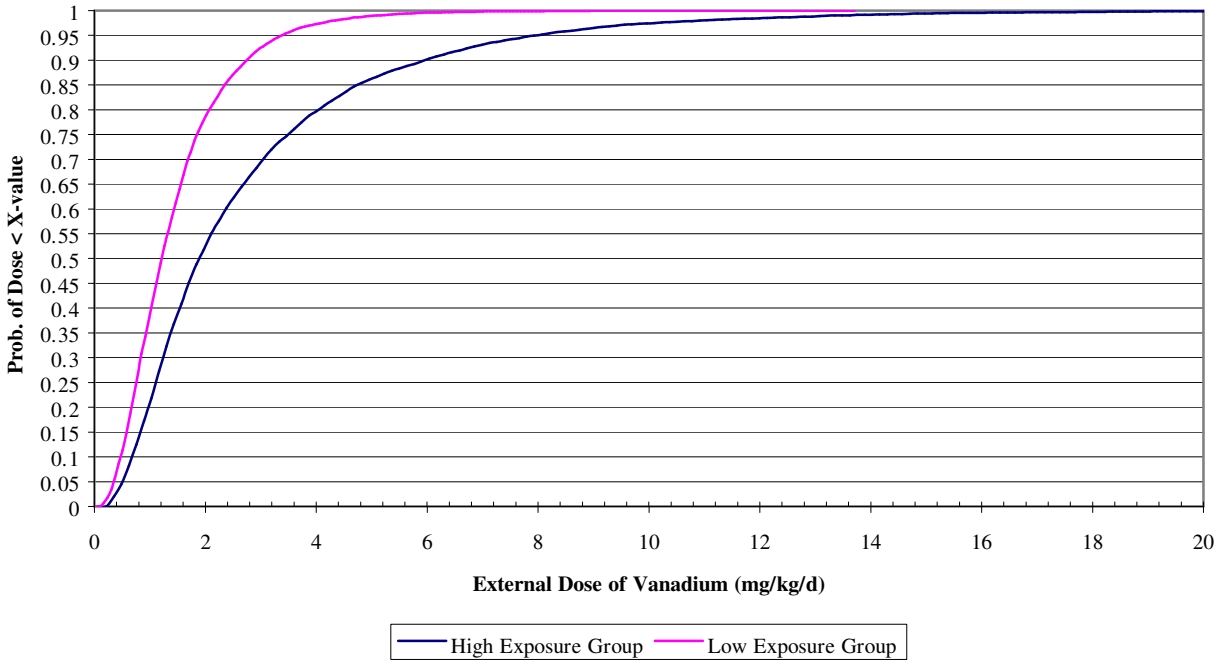

Figure 5. Distribution function for exposure dose (mg/kg bwt/d) of vanadium for adult cattle over 5 year period

CONCLUSIONS

What is the significance of the model and ultimate result? Firstly it offers an alternative to dispersion modelling and goes one step further by considering oral intake as well as inhalation exposure. Secondly, it accounts for the variability and uncertainty in the data accumulated over the five-year period. Thirdly it provides a measurement of exposure that can be compared with laboratory based pharmacology and toxicology studies. It can be used to quantify a no adverse effect level for cattle and finally the model is robust enough to use it to model smaller time segments and thus compare changes in intake with changes in biomarkers.

The project has also shown that it is feasible for a mine to manage a cattle herd on it's property within it's environmental and interested parties budget. The herd has acted as an acceptable risk communication tool since complaints by farmers have dropped off dramatically since the herd was established. The herd is acting as a useful research tool for the vanadium industry by providing information on the consequences of long-term exposure.

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SERUM ZINC LEVELS OF SHEEP AFFECTED BY ULCERATIVE BALANOPOSTHITIS AND VULVOVAGINITIS

M.P. van Aardt[1]

SUMMARY

Ulcerative balanoposthitis and vulvovaginitis of sheep is a major problem in the extensive grazing areas of the Northern Cape, Free State and Western Cape provinces of South Africa. It is mainly a venereal disease affecting the Dorper and other non woolled sheep to a greater extend. It is caused by an infection with strong evidence that Mycoplasma mycoides mycoides (Large Colony) is a primary agent in the etiology. The disease causes inflammation, erosions and ulceration of the mucous membranes of the external genitalia of rams and ewes. The nature of the lesions indicates that the integrity of mucous membranes could possibly be compromised and zinc deficiency might be incriminated.

Serum levels of zinc were determined in 17 infected and 7 healthy flocks and the levels of zinc from affected sheep (n = 147) were compared with levels from healthy sheep (n = 422). The mean serum zinc value of all the sheep was 0.946µg/ml (s = 0.274) slightly below the middle of the normal range of 0.7 to 1.3 accepted by South African laboratories. The frequency distribution of the zinc values was not normal but non parametric methods did detect that the median of zinc levels in the affected sheep were significantly higher than the levels in the unaffected sheep ($P = 0.004$). The sample of means from affected farms also showed significantly higher serum zinc level than the sample of means from farms with no clinical case of UBP ($P = 0.014$). The median values of zinc serum levels from sheep on farms affected by UBP were significantly higher than the median values from farms with no clinical cases ($P = 0.018$). The degree of lesions scored on an ordinal scale of 0 to 6, based on degree of inflammation, secondary infection, and extent of mucosal damage did not correlate significantly with the serum zinc levels, neither on cumulative samples nor on individual farm samples.

INTRODUCTION

Ulcerative balanoposthitis and vulvovaginitis of sheep is a major problem in the extensive grazing areas of the Free State, Northern-, Eastern- and Western Cape provinces of South Africa. It is mainly a venereal disease affecting the Dorper and other non woolled sheep to a greater extend. It is caused by an infection with strong evidence that Mycoplasma mycoides mycoides (Large Colony) is a primary agent in the etiology.

The disease causes inflammation, erosions and ulceration of the mucous membranes of the external genitalia of rams and ewes. The nature of the lesions indicates that the integrity of mucous membranes could possibly be compromised and zinc deficiency might be incriminated.

Trichard *et al.* 1993 described the clinical manifestation of the disease as follows:
Lesions developed 4 – 10 days after the introduction of rams to the ewes for breeding. Reluctance or complete refusal to serve, is the first sign of the disease in rams. Some animals are depressed and may stand to one side with their backs arched. If still inclined to mate, free blood discharges from the preputial opening. The skin around the prepuce is often blood –stained and haemorrhaging may occur after urinating. The prepuce is swollen, erosions occur at the mucocutaneous junction and on extrusion the penis is hot, painful to the touch and the mucous membrane may easily tear and bleed. Small, scattered erosions are visible. At times the erosions are extensive, often forming ulcers covering most of the glans and the processus urethralis. Paraphimosis may occasionally develop. This is followed by extensive trauma and soiling of the penis. In primary outbreaks up to 100% of the rams may be affected.

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Some ewes presented blood-stained hindquarters, particularly around the vulva and tail area. In Dorper sheep where the tail is docked short and the vulva is visible, the vulvar lips are swollen, oedematous and red, while blood-stained fluid may ooze from the external orifice. The vaginal mucous membrane bleeds easily when handled, particularly after vaginoscopic examination. Frequent wriggling of the tail-butt and urination is seen in some cases. Flies are attracted to the inflamed genitalia which further agitates the animals. After the acute signs have abated, scab covered ulcers are visible on the vulvar lips particularly ate the mucocutaneous junction and the ventral commissure. In the primary outbreaks the morbidity may reach 70-80 %.

MATERIALS AND METHODS

Study population

Serum from 569 Dorper sheep in the Northern Cape province of South Africa were tested, the majority originated from flocks in the Namaqualand district. Flocks of farmers that complained to the Springbok state veterinary office about UBPVV problems, during June 2001 to December 2002 were investigated. Clinical cases and comparable control cases were selected. During routine fertility examinations, a represent able sample was taken from flocks with no clinical cases of UBPVV.

Case definition

A flock of sheep where one or more of the rams is clinically infected with Ulcerative balanoposthitis, is considered as an infected farm. A ram is considered as clinically affected when on extrusion and close examination of the penis, pustules, vesicles, erosions or a red, swollen or bleeding penis is found. Non-cases would be all flocks of sheep where none of the rams on examination of the extruded penis show any of the described symptoms. No laboratory tests are available to confirm infection and clinical evaluation of lesions are the only method of diagnoses.

Sampling methods

5 to 7 ml of blood was collected from the jugular vein into vacuum glass bottles marked with the number or description of the sheep. Rubber stoppers were screened off with commercial plastic material before sealing the bottles. After complete coagulation, the serum was transferred to another marked glass tube with a rubber stopper screened off. The serum was send to the laboratory by courier in cooled containers.

Observations

Penis and vulva lesions were recorded. 45 cases were scored on a scale of 0 to 6 with reference to the degree of inflammation, mucosal damage (erosions, ulcerations), secondary infection, chronic lesions and the presence of phimosis or paraphimosis.

Laboratory analysis

The samples were analyzed by the Stellenbosch Veterinary Laboratory according to a modified version of the method described by Weisman in 1974.The laboratory was not informed about the disease status of the flocks.

Summary of method: Clotted blood samples were centrifuged at 3000 rpm for 10 minutes and the serum removed within six hours of collection and stored at –20 °C until analysis. Five millilitres of 7 % Trichloroacetic acid (TCA) was added to 1ml of serum and allowed to stand for 10 minutes. After centrifugation at 4000 rpm for 15 minutes, the supernatant was decanted and centrifuged a second time at 3000 rpm for 10 minutes to pellet any remaining protein precipitate. Copper and zinc standards were prepared in the same manner as the samples. The concentration of copper and zinc was determined by atomic absorption spectrometry.

Data analysis

Microsoft Excel, NCSS and Arcview software were used to analyze the data.

RESULTS

Serum levels of zinc were determined in 17 infected and 7 healthy flocks and the levels of zinc from affected sheep (n=147) were compared with levels from healthy sheep (n=422). The mean serum zinc value of all the sheep was 0.946µg/ml (s= 0.274) slightly below the middle of the normal range of 0.7 to 1.3 accepted by South African laboratories.

Descriptive Statistics			Standard	
Variable	Count	Mean	Deviation	Median
UBP=0	427	0.9236161	0.2797642	0.92
UBP=1	147	1.010816	0.274734	0.95

Evaluation of repeatability

30 duplicated samples rendered a mean difference of 0.12 µg /ml between the paired samples, with a variance of 0.01.The standard deviation of the 60 repeated results was 0.27. When this was taken as the limits of agreement, the percentage of agreement was 90%. A Kappa value of .71 was calculated for the repetition tests when the two sets of samples were regarded as two observers.

The distribution of the data was not normal, probably because of the large number of samples and the clustered origin of the samples.

Comparison of serum zinc levels from sheep with clinical UBP and sheep with no lesions of UBP

Non parametric methods had to be used and an analysis with the Mann-Whitney U or Wilcoxon Rank-Sum Test for Difference in Medians was performed. It was found that the median of zinc levels in the affected sheep were significantly higher than the levels in the unaffected sheep (P = 0.004).

Comparison of serum zinc levels from infected farms with levels from farms with no clinical cases

Variable	Count	Mean	SD
UBP=0	7	0.8128572	0.1759464
UBP=1	17	1.004118	0.1825534

An equal variance T test was performed. The sample of means from affected farms showed significantly higher serum zinc levels above the sample of means from farms with no clinical cases of UBP.(P=0.014) At an alpha level of 0.05, Power was 0.74.

Median Statistics		
Variable	Count	Median
UBP=0	7	0.83
UBP=1	17	0.98

Again a Mann-Whitney U or Wilcoxon Rank-Sum Test was done to analyze the difference in medians. At α=0.05, the median values of zinc serum levels from sheep on farms affected by UBP were significantly higher than the median values from farms with no clinical cases. (P=0.018)

Correlation between severity of lesions and serum zinc levels

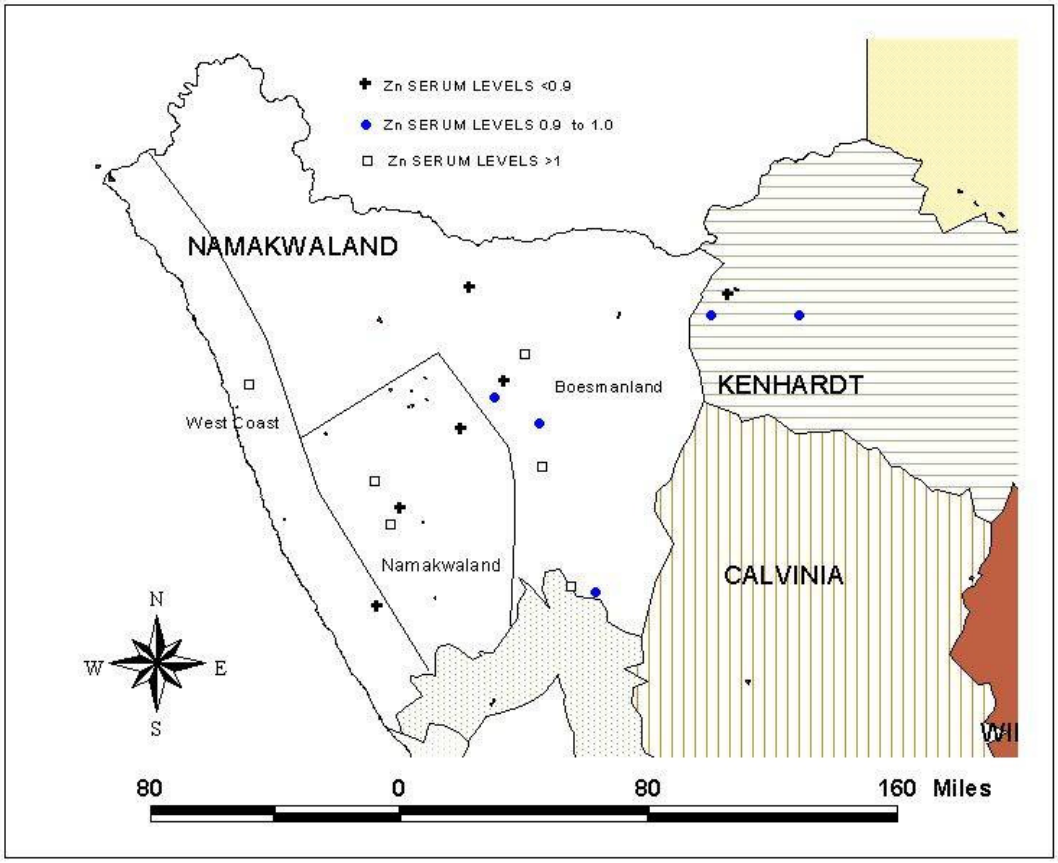
The degree of lesions scored on an ordinal scale of 0 to 6, based on the degree of inflammation, secondary infection, and extent of mucosal damage was subjected to a regression analysis against the serum zinc level, but the hypothesis that the slope was zero could not be rejected. Therefore it

was concluded that the degree of the lesions did not correlate significantly with the serum zinc levels, neither on cumulative samples nor on individual farm samples.

Evaluation of values falling outside the normal range

Values below 0.7 µg/ml and above 1.3µg/ml were examined in affected sheep, all sheep on infected farms and sheep on control farms. The proportion of sheep with a zinc level below 0.7 was significantly higher in sheep on control farms than on affected farms.(P<.01) Differences in proportions between the other groups were not significant.

Geographical considerations



The area from which the samples originate is an arid, mineral reach part of the Northern Cape Province of South Africa with extensive mining activity for inter alia zinc and copper. Three different components can be distinguished namely the west coast, mist belt with Karoo type shrubs, the winter rain Namakwaland area with a rich variety of shrubs and the dry, summer rain Boesmanland with extremely homogenous grassland. Despite the vast differences in the topography, soil composition, rainfall and vegetation, the serum zinc levels of sheep grazing the area did not reflect the differences. To a great extend the seasonal movement of sheep between the different areas would explain the homogeneity. If the higher and lower tails of the serum zinc levels are tabulated according to the locality where they were at the time of their examination, a remarkably even distribution is demonstrated:

Serum zinc level	Coastal farms	Namakwaland farms	Boesmanland farms
<0.9	1	2	3
>1.0	1	2	3

Nearest neighbor comparisons also demonstrate that high and low serum zinc levels were often obtained from sheep in the same area. 80% of mean measurements were closer to different category measurements than they were to measurements of the same category, indicating a random spread.

DISCUSSION

It is generally accepted that zinc stabilizes membranes. An antioxidant effect by protection of sulfhydryl groups in membranes is suggested. When plasma zinc decreases, it is believed that zinc dissociates from the membrane allowing association of cations leading to the oxidation to the disulfide form that does not bind zinc readily. The membrane protective effect of zinc may also involve the lipid component of the membrane. Hormone receptors and absorption sites could be affected. Intercellular binding is also reported to be dependant upon zinc. The inflammation, erosions, bleeding and ulceration seen in Ulcerative balanoposthitis and vulvovaginitis does create the impression that the mucous membranes are very sensitive.

The manifestation of UBPVV is often not explained by exposure to infection alone. A clear-cut demonstration of low zinc levels in sheep infected with UBPVV would have answered some of the discrepancies in the epidemiology of the disease. The finding that in fact zinc serum levels might be higher in affected sheep, raises the question that high zinc levels could lead to other mineral imbalances that could predispose to UBPVV.

The reliability of serum zinc determination compared with liver samples as an indication of deficiency had been considered, but it seems that both values are affected by physiological and pathological conditions. The serum samples were readily available during examination of sheep and could reflect the status at the time that UBP was evaluated.

It had been reported that serum (and liver) values of zinc does not correspond well with the clinical response obtained by supplementing zinc by the parental route. A number of farmers in the study also reported improvement and faster recovery from UBP after treatment with an injectable zinc preparation, but no controlled evaluation was done.

The skewed frequency distribution necessitated the use of non-parametric methods, and elimination of confounding factors was limited. Possible confounding factors could be different management of reproduction, feeding, mineral supplementation and many other factors on each farm. Soil and vegetation differences will have to be examined closer.

Working with the sample of means of the farms in the study improved the normality of the distribution but certain criteria were still not met. The smaller sample size on this evaluation reduced the significance of the findings.

The mere evaluation of a trend does not warrant a decrease in alpha and in fact the power could have been increased by using a higher alpha level.

Very low zinc levels were recorded on a number of control farms, but the risk of exposure to infection on these farms was unknown.

Zinc supplementation had been shown to decrease plasma copper levels in cattle. Zinc supplementation was also effective in treating copper poisoning in sheep. Therefore a negative effect of high levels of zinc in sheep, in a mineral rich part of the country, might be deleterious by inducing other mineral imbalances.

The careful monitoring of zinc levels after supplementation to evaluate the response would be valuable. Comparisons of zinc levels before and after UBP infections set in can also give an indication of the significance of zinc as a predisposing factor.

The tendency of serum zinc levels being higher on affected farms and in affected sheep was demonstrated by a number of analysis methods and can certainly not be ignored, although it should be interpreted with care.

ACKNOWLEDGEMENTS

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THE USE OF GIS IN RABIES CONTROL IN KWAZULU NATAL

R.J. Bagnall[1]

INTRODUCTION

The Province of KwaZulu Natal has been an endemic area for Rabies since 1954 when it was first introduced from Mozambique. Over the years the number of positive cases has fluctuated from less than 10 to over 300. (Fig 1)The cases are almost exclusively in dogs while other domestic animals account for 10 % of the cases reported.(Fig 2)

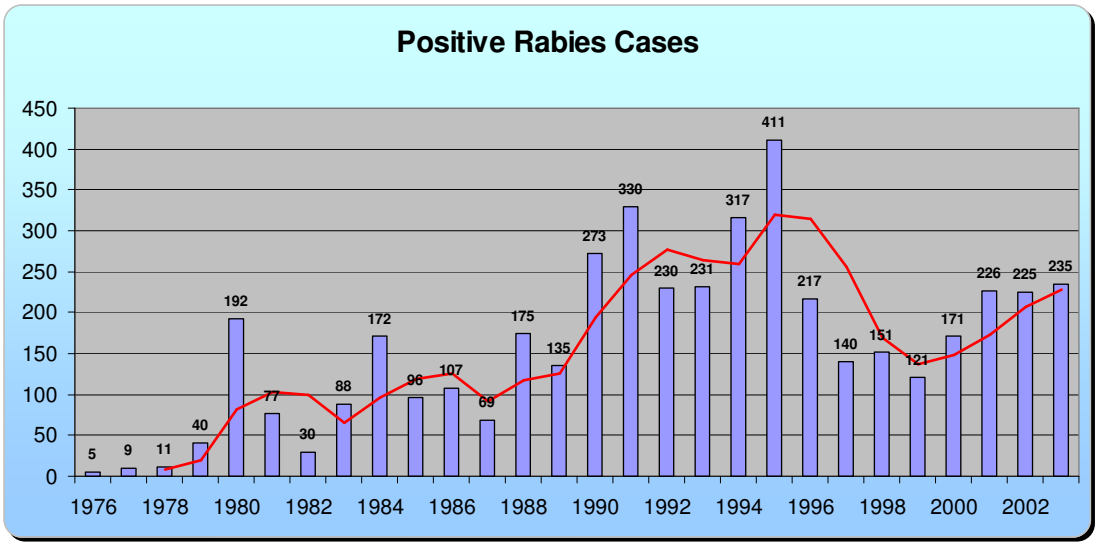

Figure 1.

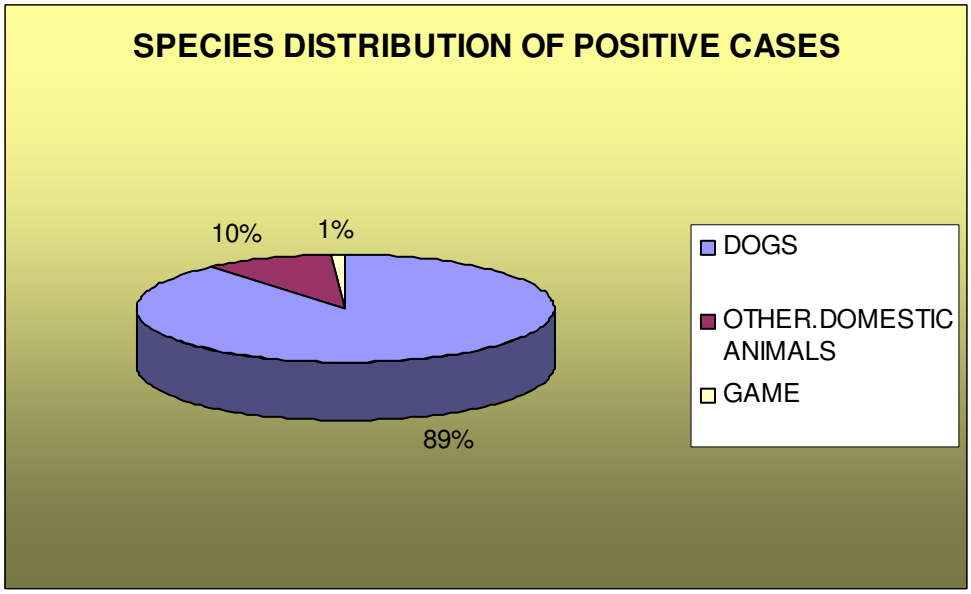

Figure 2.

After 1994 and the amalgamation of the two Provincial veterinary services, the number of cases dropped dramatically and it was thought that finally Rabies was being bought under control. However over the last number of years the number of positive cases has once again escalated and the disease has spread to areas of the province that have traditionally been free of the disease.

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Traditionally Rabies was confined to the coastal areas with ‘hot spot’ areas in Port Shepstone, Durban and Eshowe. However over the last few years the disease has spread throughout the whole province with only a few aeas reporting no cases in the last 2 years.

During 2003, a pilot trial was run in the Inanda area north of Durban in which large numbers of technicians were deployed in mobile teams which traveled all roads in the area and announced the vaccination campaigns using loudhailers fitted to the vehicles. People then bought their dogs to the side of the road and had them vaccinated. This method led to a dramatic increase in the number of dogs vaccinated and due to the large number of teams in the area, led to increased security as well.

This pilot trial proved to be a success and was then rolled out to other ‘hot spot’ areas.

This method of vaccination is now being used in all areas of the province with varying levels of success.

During 2004 the areas vaccinated using this method were revaccinated and it is hoped that this second vaccination will eliminate Rabies from these ‘hot spot’ areas.

The staff of Veterinary Services, KwaZulu Natal, formulated a vision in which the incidence of Rabies must be reduced by 70% by the year 2006 and completely eliminated by 2010.

The use of these mass campaigns is however very costly and requires large amounts of manpower and logistics. In an era of shrinking budgets for Veterinary Services, the sustainability of such campaigns is in doubt. It is therefore essential to monitor the results of these efforts in order to convince senior management to support such efforts financially and logistically.
The use of basic GIS techniques can greatly assist in this effort.

USE OF GIS IN RABIES CONTROL

Mapping of positive cases

Rabies samples are submitted to Allerton Laboratory by State Veterinarians, Private Veterinarians and other role players. Results are forwarded on the same day to the sender, local State Veterinarian, Health Department and other role players. In positive cases, the local State Veterinarian investigates the case and supplies the Epidemiology office with the geographical coordinates of the case as well as details of any human contacts. The local Communicable Disease Coordinator from the Department of Health is also involved and ensures that all human contacts receive the correct post exposure treatment.

The Epidemiology section then plots the case, checks the coordinates and then enters the data onto the Rabies Database.

A monthly report is produced in which all positive cases are listed and mapped. This monthly report is then circulated to all role players.

The mapping of positive cases also assisted in identifying the ‘hot spot’ areas which were then targeted with mass vaccination campaigns.

An attempt was made to try and map the spread of the disease but without any success. The disease appears to be too sporadic at present, perhaps as a result of under reporting or by the movement of infected dogs by road to new areas. This aspect of Rabies control needs to be further investigated and expanded so that predictions could be made in future as to the spread of the disease.

Mapping of vaccination campaigns

One of the major shortcomings of our Rabies control is that we have limited spatial information on the vaccination campaigns. Vaccination figures are reported per magisterial district, very often only a portion of the district will have a Rabies problem. This makes it very difficult to compare areas vaccinated to the prevalence of Rabies in that specific area.

During the vaccination campaigns held in 2004, an attempt was made to map the vaccination area.

A small pilot area was selected and a basic map produced showing all known roads in that area. It has to be remembered that not all tracks used by the vaccination teams are shown on the GIS

database of roads. After discussion with a number of technicians and owners it was estimated that the longest distance an owner would walk with their dogs was 1 km. This distance will obviously differ according to the terrain in the area.

The roads were then buffered by 1km and the buffers combined. This then gives an estimate of the coverage achieved in the area under question.

The results of this exercise showed that the area selected was very well covered and that at least 90% of the population were able to get their dogs vaccinated. This particular area has a high human population density and consequently a large road network.(Fig 2)

However, when the same exercise is applied to an area in the middle of Ingwavuma district then it shows that the area covered is less than 10 % if the roads alone are used.

Thus every area will have to be approached in a different manner.

A new project that is planned to start soon is to identify the vaccination areas using 1:30,000 colour aerial photographs. This will enable more accurate mapping and will also enable the team supervisors on the ground to ensure that all areas are covered.

Positive cases will also be able to be plotted on these aerial photos, leading to a much more focused vaccination strategy being applied. This will become more and more important as the prevalence of Rabies drops to lower levels.

In the 'hot spot' areas it will become important in future to plot the negative cases as well to ensure that a drop in the number of positive cases is not due simply to a lack of samples being submitted.

Dog population survey

The most important piece of data that is missing from database is an accurate estimate of the dog population in the province. Without this, the vaccine coverage in an area cannot be estimated.

Various attempts have been made in the past to link the dog population to human population. This led to very large variations between areas and can at best be used as a very rough guide.

During the present vaccination campaigns, a survey was conducted amongst the dog owners presenting dogs for vaccination and it was found that on average there are 3 dogs per household. This figure can be used as a rough guide but as it only included dog owners it cannot be used to estimate the total population.

Together with the Department of Health Epidemiology section, three areas have been identified for a pilot trial. A rural area, a peri-urban and an urban area have been selected. The selection was made on the new municipality ward boundaries.

In a combined survey, staff from both Departments will then conduct a statistically valid survey within each area. From this and using the known human population figures and number of households in the area, the total dog population can be determined with an acceptable degree of accuracy.

These figures will be confirmed by conducting similar surveys throughout the province.

It is hoped from this survey to once and for all determine the overall dog population in the province and this will enable the vaccination teams to set a target for every area covered.(assuming that a 70 % coverage is necessary for Rabies control)

A spin off from this survey will be more details on the structure of the dog population which could also help explain the present Rabies epidemic.

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POSTERS

THE CLINICAL SIGNS, PATHOLOGY AND PATHOGENESIS OF CHRONIC ENVIRONMENTAL VANADIUM POISONING IN CATTLE

B. Gummow[1], C.J. Botha & S.S. Bastianello

South Africa is one of the largest vanadium producers in the world. Large areas of the country are rich in vanadium and the country has an active vanadium mining industry. Vanadium poisoning has long been an occupational health risk within the mining industry. Mines and cattle farmers are often found in close proximity to one another. Sporadic incidents of vanadium poisoning in cattle have been reported in South Africa over the last 30 years. Little work is being done elsewhere in the world on the impact of chronic exposure to vanadium pollutants, particularly in cattle.

During the period 1990 to 1993, an outbreak of vanadium poisoning in dairy cattle was investigated by specialists from the Faculty of Veterinary Science, University of Pretoria (UP), Onderstepoort Veterinary Institute (OVI) and Institute of Soil Climate and Water (ISCW), South Africa. During the course of this investigation, 60 dairy cattle were evaluated clinically and by means of chemical pathology and full necropsies were carried out on 17 cattle that died. This work progressed into an experimental trial using calves (n=16) from the contaminated farm to evaluate the use of CaNa2EDTA as a potential treatment for vanadium toxicity in cattle and allowed the collection of further data on the clinical signs and pathogenesis of vanadium poisoning. A five year field trial in another part of the country was then set up comprising a sentinel herd of 30 Brahman Cross cattle. These have been run as a commercial herd and experimental cohort on a vanadium mining property since 1999. One of the objectives of this study was to provide a long-term animal sentinel model for the vanadium mining industry that would act as an early warning system for problems associated with complex pollution exposures. These animals have been monitored by the UP for four cattle generations and further data has been accumulated on the long term effects of chronic vanadium exposure in cattle on production and health. These data include various alternative biomarkers for monitoring vanadium exposure and tissue levels of vanadium at slaughter, which were used to assess the public health and food safety aspects of chronic exposure of cattle to vanadium. During this study 17 calves developed symptoms at various intervals and despite attempts to treat them, 8 died and 9 had to be euthanased. Necropsies were performed on these animals and tissue samples were taken for vanadium analysis.

This poster summarises the main clinical and pathological findings of nearly 13 years of research on vanadium poisoning in cattle. It also provided some insight into the pathogenesis of this disease in cattle. Young calves are by far the most susceptible age group to exposure and develop signs concomitant with malabsorption related hypoproteinaemia and immunosuppression. It is thought that the pathogenesis is a combination of physical damage to the gasto-intestinal tract and biochemical damage. Physical lesions included a mild to moderate granulomatous and eosinophilic enteritis and typhlocolitis, moderate villous atrophy characterised by fusion and stunting of villi and dilatation of the lacteals, and mild parakeratosis of the ruminal and omasal mucosa. Biochemical lesions are associated with vanadium's ability to modify the activity of a number of enzymes, including NaK-ATPase, which is important in muscle contraction, tyrosine kinase, which is located in growth factors, oncogenes, phoshatases and receptors for insulin (Berner *et. al.* 1989, Nriagu, 1998). Vanadate and vanadyl ions also inhibit Ca and Mg-ATPase, which are important in synaptosomal membranes in nervous tissue as well as in facilitating muscle contraction (Nriagu, 1998).

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RESULTS FROM A PRELIMINARY SURVEY TO DETERMINE THE PREVALENCE OF *ORNITHODOROS* TICKS AND AFRICAN SWINE FEVER VIRUS IN GAUTENG

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African swine fever (ASF) is a devastating haemorrhagic disease affecting domestic pigs. Outbreaks of the disease can cause 100 % mortality and since no vaccine is available, control relies on zoosanitary measures and eradication during outbreaks. In Africa the disease is maintained in two cycles, an ancient sylvatic and a more recent domestic pig cycle.

African swine fever is endemic to certain areas of South Africa where infected warthogs and soft ticks of the *Ornothidorus spp*. (tampans) occur. A boundary has been determined that divides the country from North to South going through the provinces of KwaZulu-Natal, Mpumalanga, Limpopo and North West, where the regions north of the boundary are deemed infected and the rest of the country is free of ASF. This boundary was determined by historical outbreaks of ASF and the occurrence of tampans.

Although Gauteng province is in an area classified as free of ASF, regular surveillance has been carried out along the northern provincial borders to monitor the status of tampans and ASF virus in this region. Biannual (Feb/Mar and Nov/Dec) visits to purposively selected farms along the border were conducted between November 1997 and March 2004. A total of 107 visits were conducted to 27 farms during this period. During each visit, warthog burrows were sampled for the presence of tampans. When tampans were found, they were tested for the presence of ASF virus.

Tampans were found on 13 of the 27 farms (48%) and virus was isolated from tampans on 5 of these 13 farms (38 %) during the 7-year period. There was no period when tampans were not found on at least one of the sentinel farms, however virus was only isolated in February 1998, December 2002, February 2003 and March 2004.

The *p72* gene of the isolated viruses was sequenced and compared to viruses previously characterised in southern Africa and well as in East and West Africa. The Gauteng strains were all closely related to each other and to viruses isolated from Pienaarsriver, Ellisras and Rustenburg prior to 1996. These viruses were all distinct from those obtained from the rest of sub-Saharan Africa.

The findings indicate that conditions are favourable for the maintenance of tampans along the Gauteng border and that virus is present at least on occasion south of the historically free zone. This could have serious implications for farmers in Gauteng and it is recommended that a more intensive survey be carried to determine whether these results have bearing on the current ASF boundaries.

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OVINE ENZOOTIC ABORTION: DISEASE CHARACTERISTICS AND ASPECTS OF VACCINATION CONTROL IN SOUTH AFRICA

B.F. von Teichman[1] & T.K. Smit

Ovine enzootic abortion (OEA) is caused by an obligate intracellular bacteria *Chlamydophila abortus* (formerly the ovine subtype of *Chlamydia psittaci*). *C. abortus* is widely distributed throughout the world causing abortion and foetal loss in sheep, goats and cattle. The majority of aborting ewes are young animals, although sheep of all lamb-bearing ages are potentially at risk of infection. *C. abortus* infection occurring for the first time in non-vaccinated pregnant ewes may cause 30% to 60% lamb mortality. It is common that about 5-10% of ewes abort annually in vaccinated flocks, as well as, where the infection is established as an enzootic disease. *C. abortus* infection resulting in abortion leads to effective immunity in affected ewes. Transmission to humans and spontaneous abortion in women caused by Chlamydia has also been documented. In South Africa, sporadic cases and outbreaks of OEA in sheep are reported in many sheep rearing areas of the country. The economic importance of the disease on the ruminant industry however, cannot be assessed due to the lack of epidemiological data.

Aspects of the signs and transmission of the infection, diagnosis, the immune response and vaccination control will be highlighted.

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JOHNE'S DISEASE: GUDAIR VACCINE TRIAL

M. Sinclair[1], & P. Koen

Johne's disease has a crippling economic effect on sheep farmers in the Overberg and Swartland regions in the Western Cape province. Some farmers report an annual cause specific mortality rate of up to 14%. In this region the disease progressed in such a manner that whereas in 1996 and 1997 predominantly older sheep (full mouth and older) were affected, currently even sheep younger than a year show typical symptoms of the disease.

The Directorate: Veterinary Services, Western Cape province, together with Virbac Animal Health, conducted a vaccine trial on 3 affected farms in the problem areas. The vaccine (Gudair®) is currently in use in Australia, New Zealand and Spain, where its efficacy in reducing mortalities has been proven. It is known that Gudair® vaccine causes lesions at the injection site in a significant number of animals. The purpose of this trail was to evaluate the severity of these lesions in herds with high infection rates and herds with a lower level of infection. In addition to measuring and evaluating the lesions, the weights of the sheep in both the sample and control groups were recorded.

150 Sheep on each of the three farms (50 adults, 50 six-months old, and 50 lambs of approximately 4 weeks of age) were vaccinated. The control group consisted of 150 additional sheep within the specified age groups. During the 300 days of the trial, significant lesions were recorded in 98% of animals with 14-26% showing abscesses or open wounds at 30 days post vaccination. The subsequent reduction in lesion size was variable and unpredictable. The average weights of the animals showed fluctuations according to the drought situation, but there were no significant differences between the sample and control groups.

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COMPARISON OF SOLID PHASE COMPETITION ELISA AND LIQUID PHASE BLOCKING ELISA FOR DETECTION OF ANTIBODIES TO THE STRUCTURAL PROTEINS OF THE SAT TYPES OF FOOT-AND-MOUTH DISEASE VIRUS

J.J. Esterhuysen[1], O.C. Phiri, B.B. Botha, H. Jones & W. Vosloo

The internationally accepted tests for the detection of antibodies to the structural proteins of foot-and-mouth disease virus (FMDV), as prescribed by the Office International des Epizooties (OIE), are the virus neutralisation test (VNT) and the liquid-phase blocking ELISA (LpBE). While the VNT is concidered to be the "Gold Standard," the LpBE is more commonly used for the routine screening of large numbers of sera. However, studies have indicated that the LpBE can give false-positive results that may vary according to the animal population under consideration, and can be as high as 18% in stressed animals. Due to these problems, a solid-phase competition ELISA (SpCE) was developed by Mackay *et. al.* (2001), and compared with the LpBE and the VNT. The specificity of the SpCE was considerably higher than the LpBE and comparable to that of the VNT. Furthermore, the SpCE is easier to use and more robust and therefore offers an improvement for FMDV antibody detection. The test has now been proposed as the preferred ELISA by the OIE.

The test is currently not available in kit form, and has to be developed and validated for the SAT serotypes prevalent in sub-Saharan Africa. Test conditions have been optimized by cross titrations of the reagents used in assay. These include the antigen, the guinea-pig typing serum and the conjugate. The conditions for the test have further been investigated using a panel of known control sera. Initial experiments indicate that less variation occurred in the results of the SPcE with different concentrations of reagents, than was the case with the LpBE. This indicates the robustness of the SpCE.

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SURVEY OF SMALL SCALE DAIRY FARMERS IN CENTRAL NORTH WEST PROVINCE USING PARTICIPATORY RURAL APPRAISAL

P.J. Sebei[1], L. Prozesky[1] & C.M.E. McCrindle[1]

The Department of Land Affairs has revised its Land Reform Programme to support sustainable rural development policies and interventions. Its focus will shift over the medium term to the implementation of an integrated programme of land redistribution and agricultural development. The programme is designed to provide grants to previously disadvantaged people to access land, specifically for agricultural purposes. Dairy farming has been identified as a priority by the North West Province (NWP) of South Africa, particularly in the Central Region, as it has the potential, not only for job creation, but also as a sustainable source of high quality protein for rural communities.

There are 791 dairy farmers in the province (excluding those who produce for home consumption and local sale). National statistics show that 46% of producers produce between 0 and 500l/day but contribute only 9% of total production. Therefore, many of the milk producers that are classified as commercial are in reality also small-scale producers, as many of these herds will number less than 20 cows in milk. With the current land redistribution program many smaller units are being created and semi-intensive or intensive enterprises have to be considered. The way in which the production system is managed plays an important role in profitability in terms of input costs and milk production at lower volumes.

After purposive selection of 15 farmers that met the criteria of the North West Province and project leader, a workshop was held with the farmers and their extension and animal health officers to discuss and prioritise short and long-term objectives using facilitated focus group discussions. A structured interview was developed from the findings of the workshop. This was followed by farm visits. In addition to the structured interview, informal interviews were conducted with farmers, their families and beneficiaries. Observations were made and photographs taken to record the available infrastructure, management, farming system and cattle breeds used.

The interviews and on-farm observation revealed serious deficiencies in the skills, knowledge and attitudes of small-scale dairy farmers. Very few farmers had received training at any level in dairy management, and it is obvious that there is a need for training if small-scale farmers are to make a success in this field. On most farms the system of milking and calf rearing was closer to the traditional management of communal cattle than specific management for dairy production Most farmers did not feed suitable concentrates and roughage to their cows, and this deficiency was reflected in the low milk yields. Milk hygiene was found to be sub-optimal and was probably partly responsible for the poor keeping quality of milk. Few of the farmers had milk-cooling facilities or bulk tanks and most sold to the informal market. Only one of the farmers used a milking machine.

A serious deficiency on most of the farms was the paucity or absence of records. Poor records make it impossible to assess the efficiency of an enterprise and to identify the constraints affecting profitability. However, the average prices received for milk were high: R4:00 per litre is approximately twice the price received by commercial producers. At these prices there is a good potential for these farmers to make a profit and improve food security in outlying areas if their production capacity and milk hygiene is improved through training.

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THE ROLE OF EPIDEMIOLOGY IN THE REVISED CURRICULUM FOR THE *ND: ANIMAL HEALTH* AS OFFERED BY UNISA(TSA)

A.J. Higgo[1] & C.M.E. McCrindle[2]

The National Diploma (ND): Animal Health was presented with inception 1991 at the former Technikon South Africa (TSA) on special request and in close co-operation with the national and provincial Directorates of Veterinary Services. This diploma is aimed at training animal health technicians competent in assisting state and private veterinarians in the following: applying animal health care, disease control and management techniques in the prevention and control of animal diseases.

The former TSA, now University of South Africa (UNISA), is the sole provider of this course in Southern Africa. Since its initial introduction, stakeholders have regularly reviewed the diploma, which has subsequently been re-curriculated repeatedly to meet the changing requirements of the profession within industry. The last revision of the curriculum was agreed on during various re-curriculation meetings and workshops with stakeholders in 2003 and the new course will be offered from 2005. As one of the major changes in the revised course compared to the existing curriculum, Epidemiology which used to be a one level subject has been extended to a three years offering to facilitate learning and to emphasize the importance of this subject. Syllabus content and outcomes of all three levels have been redefined and the present course was tailored according to the needs of industry with regard to the professional needs of animal health technicians. This poster will highlight content and outcomes of the revised syllabus and put it in context with the complete new curriculum of the diploma.

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