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OPENING ADDRESS

Dr Bob Swanepoel

Consultant Virologist, National Institute for Communicable Diseases

level 4 infection : Crimea-Congo Fever. only treated in level 4 facilities. On the back of the person is a ~~pollen~~ air filter. Biosecurity level 4 laboratory, work with gloves - put it in a line - move from one side to another. Viral haemorrhagic fevers classified according to transmission & hosts.

Viruses:

	Reservoir hosts	
Arenaviruses	– rodents	} not in S. Africa.
Hantaviruses	– rodents	}

	Vector	
Yellow fever.	~~or~~ mosquito	man / monkey.
Dengue.	"	man (monkey.)
RVF	Ticks	
Congo		Bats ?
Ebola		Bats ?
Marburg.		

Congo fever: Bont poot ticks. Southern hemisphere & Europe, Indian, Africa - disease is hyperendemic, Hyalomma ticks. The larvae feed on rodents / rabbits. The adult tick likes big hosts: cattle, humans. Smaller then impala - no antibody: Giraffe, Rhinos - bite so antibodies. Live in dry areas. Do not hang on the grass. Do not usually bite humans. It is not a disease of cattle: when an animal is young it becomes infected and immune. Can get adult cows viraemic if brought into contact with ticks for first time. Blood contamination hands can be a problem. You see it in the carcasses but pH changes kill the virus. Fresh blood is dangerous. Incubation period short. Sporadic fever: up & down. Very

characteristic picture Exposure to ~~blood~~ body fluids - incubation is 1-3 days. 5-6 days if exposed to blood. Extremely sudden onset, extreme headache, conjunctivitis, rash on trunk. Bleeding tendency. Bleed under skin. Vomit blood. DIC very quickly. All coagulants used up & bleed massively. Where injected - huge haematoma. Fibrinogen: early on the fibrinogen is used up. These people die. Coagulative necrosis of liver. Immune haemorrhage of the liver. ~~Needle Stamper in Africa~~ - ~~used same needles~~

~~Clin~~ Clin Path: Leukopaenia / leukocytosis >50% reduction in 3 days

Thrombocytopaenia.

Liver function tests. Doctors/nurses die so w.b.

3000 outbreaks over a 20 year period. 4600 cases.

Consequences of misdiagnosis: 1 person in a ward: costs R1.00 m. per day because ward is emptied. If not moribund on day 9, not congo fever. All over South Africa.

RVF: Strongly associated with rainfall. Mosquitoes ↑↑↑.

Outbreak in Somalia 1997. Saudi Arabia - wadis with flood plains. ↑↑↑ rain in 2000 → outbreak due to ↑↑↑ mosquitoes. 220 cases and deaths.

Ebola virus:

Needle Stamper: epidemics in hospital. Outbreaks in Africa. What do people eat? Everything. Crocodiles, bats, monkeys, snakes, insects. Man meets forest. Gorilla carcasses implicated. Caught insects. Virus in blood and faeces of bat.

Monbeng: monkeys imported from Africa. Some tourists in contact with bats. (Outbreak in SA). In 1989 in N.E. Zaire. Underground diggings. Pumping out water. Kept coming back. Went on for 2 years. Linked to mine. Once flooded, outbreaks stopped. Known since 1887 in area. Tens of thousands of bats in the mine. Suspect bats are carriers.

PAPERS

One Medicine:

Esther Schelling, Dept of Public Health and Epidemiology, Switzerland (See slides sent by e-mail). Asking for joint approaches - in traditional societies, healers deal with human and animal health. Karl F Meyer worked with Theiler before finding the first VPH course in California.

"Agroecosystem health" investigation of agricultural systems in a holistic view encompassing ecology, economy and health. "Ecosystem approach to health". Relationship between various components of an ecosystem to define and evaluate priority determinants of human health → sustainable ecosystem management of the ecosystem. Gaia hypothesis. livestock keepers are susceptible to exclusion from markets, primary veterinary and health care. Who are the most vulnerable? Why do some fare better? Look at livlihood frameworks. Burden of human disease: DALYs. Not yet established for zoonoses.

zoonoses: - opportunistic/emerging
- Poor more exposed: hygiene, debasy
- Classical zoonoses linked to livestock
- Food borne diseases
- Breakdown of public infrastructure
- Misdiagnosis (80% of animals with serotype for Q fever in Chad). (Q fever 40% of malaria cases in Mali)
- Information sharing, cost effective zoonoses.

Interdisciplinary collaboration: Tb. link with political and social sciences - outbreak of Brucellosis in livestock after: transsectorial health economic assessments of zoonosis contal Zinsstag et al 2005.

Roth et al 2003. Information is demand - driven (Becher et al, 2004)

USE OF SIMULATION MODELS TO ASSIST PUBLIC HEALTH POLICY ON ZOONOTIC DISEASES

P.S.A. Woods[1], D. Hartley & L. Hungerford

ABSTRACT

The spread of important zoonotic diseases due to the breakdown of veterinary control in some African countries means that veterinarians, wildlife experts and doctors have to collaborate and agree on suitable strategies for managing diseases, especially those that are likely to have transboundary spread. Epidemiologic compartmental models allow dynamic investigations of the importance of various risk factors in disease control. Re-running the model with changes in the likely values can indicate important control points, as well as areas where research is needed. Vensim® (Ventana Systems, Inc.) is freeware used to produce dynamic simulation models. This program is quickly learnt, easy to follow and enables various groups with different perspectives and aims to work together to determine likely outcomes of alternate solutions. The use of Vensim to produce models for rabies control with different primary aims is demonstrated; i) control methods to reduce human exposure in rabies-endemic areas, and to contrast this with; ii) a system whose primary aim is conservation of wilddogs in areas of sparse human population. The models can be used to explain why some control strategies have not been successful, and to provide likely results where it would be irresponsible/unethical to conduct the trials.

INTRODUCTION

Epidemiologic modelling is an important tool for conceptualizing, analyzing and testing assumptions about disease in domestic and wild populations. Risk factors can be evaluated for impact on disease control, and areas identified where research is needed. However, developing a realistic model about a disease or species where one is not an authority requires gaining much background information. There is also a high possibility of inclusion of an incorrect value or pathway resulting in loss of credibility for the whole model.

A number of different software packages can be useful for disease modelling. The most traditional allow writing and solution of mathematical equations to capture disease dynamics. Unfortunately, these often limit participation in the modelling process to those who have extensive training in calculus and a knack for equation solving. Another approach is to construct "picture-based" models that represent the main components of the population and disease processes. These can be very inclusive, but a drawback is that they can easily become very complicated and "blow everyone away". This does not encourage "ownership" of the model by the audience. Rather, models should be condensed down to emphasise the salient points when speaking with policy makers, but the detailed potions, especially the mathematical relationships, should be explicitly captured and available if needed to improve understanding or to increase the credibility of the model. It is critical that the modeller consider the answers required by his or her clients, and also our client's clients. They each will have questions that can be answered by the model, and these elaborations could be left in, and other "not-so-interesting-to-these-clients" portions shown in less detail. All the values used in the model can be shown & printed out, thus increasing the transparency of using the models. Also useful graphs and curves can be produced for illustrative purposes.

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Vensim® is free and easy to use commercial software package, from Ventana Systems, Inc., http://www.vensim.com. When developing a model with a group, it can be used to first make a schematic diagram of the problem, and decide which factors should be considered. The equation editor assists in developing equations using those factors that were decided to have an influence. Simulations can be run to generate predicted outcomes, changing values of inputs. It is easy to see the effects of these changes on the outcomes.

The use of this easy to understand and highly visual program to develop a model encourages input from disease experts and also those who will be implementing the changes in the field (who are often not mathematicians), and a more satisfying and useful model will be result. This collaborative process will produce realistic values and limits to include in the model, and once the model is run these collaborators will then be able to add their feedback on where the results are similar or different from those observed in the field. Participation in model development may also increase "buy-in" to implement those changes and control measures suggested by the model.

The values that are agreed during discussion of this relatively easily understood Vensim-built model could also be used to develop the same model in a more mathematically robust program, such as R (http://www.r-project.org/), which is also freeware but with a mathematical interface that is unlikely to invite input from most veterinarians! However, the simple elegance of the mathematical approach may yield new insights into general principles of disease control that would not be realized in the Vensim representation.

METHODS

To illustrate this approach, a relatively simple model, constructed in Vensim to show rabies transmission and control in dogs, is adapted for two different situations with alternate endpoints; i) rabies control in an high-density human population with a large number of free-ranging dogs where the primary aim is to decrease human and pet contact with rabid dogs, and ii) rabies control as one part of a veterinary programme to be instituted in a conservation park with a wild dogs breeding programme *(Lycaon pictus)*. The purpose of each rabies control model is different and alternate control methods are options for each situation.

Basic Model

The basic conceptual model of the rabies transmission cycle in dogs was developed as modified SEIR (Susceptible, Exposed, Infectious, Recovered) model. At any given time, dogs are thought of as susceptible to infection with rabies virus; exposed, infected and incubating the virus; infectious and showing frank disease; or recovered with immunity. Death is possible in each of these possible states; new births are only allowed into the susceptible and immune "compartments". Transitions between these epidemiologic states are allowed as time evolves and are dependent upon such quantities as contact rates; incubation periods, recovery rates, natural and disease-related mortality rates; and the rate at which births occur in the population under consideration.

The rates of transition between the different states will be population-dependent ,just as they are in the nature, and challenge the user to collect and use data meaningful for his/her unique application.

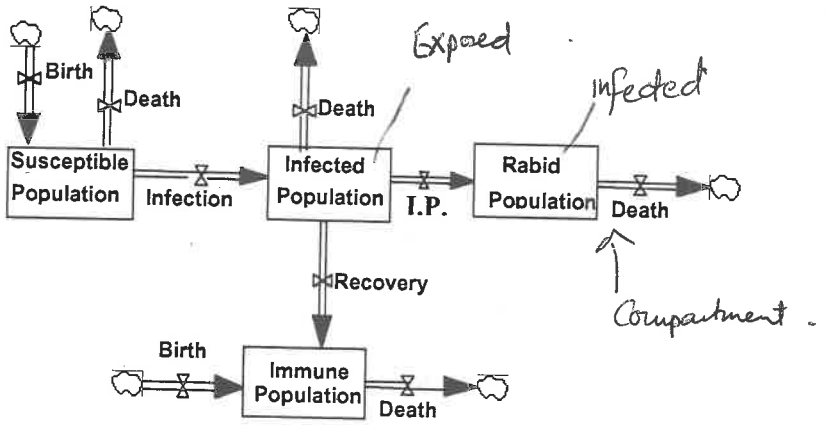

Figure 1. Basic epidemic model showing "compartments" of individuals and "flows" between compartments.

Urban Dog Scenario

For the urban dog situation, factors to be considered could include vaccination campaigns using injectible vaccines together with methods of identifying the immunized animals. Also neutering, spaying and culling may be options to decrease the population of susceptible animals.

Wild Dog Scenario

For the wild-dog conservation situation, additional factors should be considered such as the minimum population required to sustain rabies, changing habitats to decrease contact with other species, and methods of vaccine distribution which could decrease stress on the wild dogs e.g. oral vaccines. The dispersal patterns of wild dogs may be of great interest as they can dictate the month of vaccination, and the spread of disease. Weekly update - eg so model is dynamic.

Example of scenarios

One attractive feature of the Vensim software is the ease with which the user can investigate the effect of uncertainty or variation in individual parameters upon model output. Vensim offers the ability to adjust all parameters with a slide bar-like, click-and-drag feature. In this way, the user can literally see how robust a particular result is with respect to changes to the parameters of the model.

CONCLUSIONS

In conclusion, by constructing a simple model of rabies in domestic and feral dogs, a clear and present veterinary public health issue throughout Southern Africa, we have been able to (1) cast a working hypothesis (the basic epidemiology of the disease as well as possible control and intervention measures) in quantitative terms; (2) communicate that hypothesis to an audience including veterinary health professionals, policymakers, and basic scientists; (3) identify key data that are needed to understand rabies transmission and spread in a given locale or region; (4) analyze our working hypothesis; and (5) investigate the impact of uncertainty in the model upon our conclusions. These steps represent a paradigm for evidence-based decisionmaking. Mathematical and computer models are powerful analytic tools for supporting such activities.

SOME PROBLEMS ASSOCIATED WITH DETERMINING THE BRUCELLOSIS AND TUBERCULOSIS STATUS OF DAIRY CATTLE AND THEIR PRODUCE IN KWAZULU-NATAL

K.D. Perrett[1]

BACKGROUND

From mid 2003 the Epidemiology unit has attempted to map the Brucellosis (serology) and Tuberculosis testing undertaken by the Kwazulu Natal (KZN) State Veterinary Services in KZN. In the course of gathering this information, it soon became clear that, as far as Brucellosis serology testing was concerned, there was a difference between what the lab was reporting as "positive" and what the State Vets (SV's) were dealing with on the ground. In 2004 for example, Allerton Laboratory processed 71554 Brucellosis serum samples of which 535 were classified as "positive". In the same year the SV's reported that they were working with a total of 5 positive CA commercial herds and 5 positive diptanks in the communal area. All these herds or diptanks typically involved a few animals at most.

The mapping exercise also revealed significant geographic "holes" in the SV testing, some of which coincided with the location of major dairying areas in the Province. Private Veterinarians service these farms with the SV's only involvement usually being the issuing of CA/TB certificates based on testing organized by the Private Veterinarian. The KZN Veterinary Services have thus largely lost direct contact with a large number of farms, which are the same farms that supply the majority of dairy produce for local consumption and for export from KZN, both internationally and to other provinces within RSA.

This presentation will deal only with this latter aspect but it is obvious that sooner rather than later, we also need to address the question and develop a protocol of how to deal with laboratory "positive" animals in communal areas where animal movements are even less structured than in commercial areas.

THE MAPPING PROCESS

This, in theory, is a relatively simple exercise. Each State Vet, in the course of his/her normal duties, submits a standardised monthly report in which is detailed the activities undertaken for the month. A new section was added for Brucellosis and Tuberculosis testing in which the farm/Diptank is identified by name and the GPS co-ordinates recorded. These are then simply transcribed into an Arcview database and mapped.

In the case of the Dairy Farms, Clover supplied the GPS co-ordinates of all their farms in KZN, as well as of some farms supplying the smaller dairy companies (Stonelees etc). The remaining known dairy farm's co-ordinates will be obtained either by SV field staff, through Eskom or from the dairy company they supply.

The maps below clearly illustrate the lack of SV involvement in CA and TB testing in KZN's dairy production areas.

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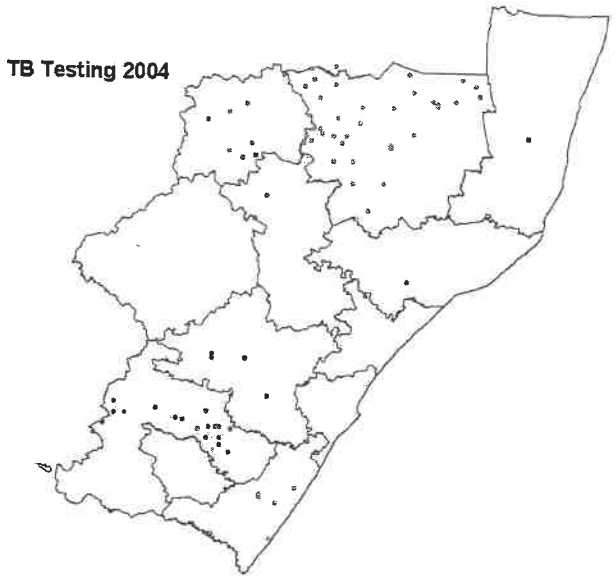

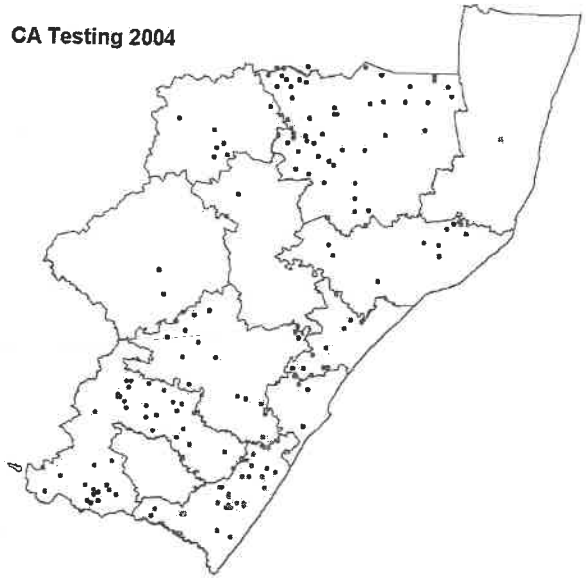

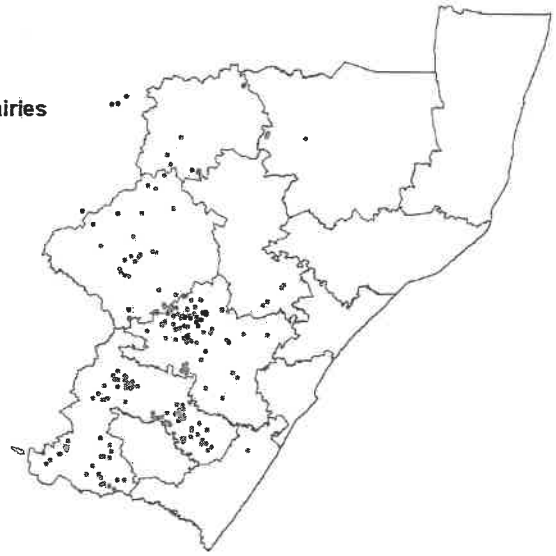

THE CHALLENGES

Problems started to emerge when some dairy companies decided to export product. I will attempt to separate the problems into a number of different areas moving from the farm to the consumer/exporter, but in reality they are all interconnected to a greater or lesser degree and revolve around verification, traceability, certification and standardization.

a) Milk Parlour registration

At the moment in KZN, the Health Department registers the milk parlour on the farm in the farmer's name. This registration process involves a check on the water quality, a site inspection based on the Department of Health regulations, and the farmer needs to provide a valid CA and TB certificate and complete an application form. Thereafter, apart from an "annual inspection" by the Health Department, the registration remains valid until the farm changes hands or changes legal status. Thus, in some areas of KZN where the KZN Health staff is stretched thin on the ground, farms theoretically could have valid parlour registrations for the whole working life of a farmer, based on their original application. From a practical point of view however, it is quite possible for farms to supply dairy produce for extended periods of time without any physical inspection of their facilities being undertaken. This gives rise to questions involving the certification of the origin of the milk. Another interesting aspect to be highlighted here is the differences in the legal requirements of Veterinary Public Health and the Department of Health for these facilities especially in terms of export requirements.

b) Milk Ring Tests

Farmers supplying milk to dairy companies are obliged to have a monthly MRT done on their milk and to annually supply the company with a certificate stating that their herd is CA free. The State Veterinarian has to issue this certificate based on the results of the MRT's. As a service to the farmers and no doubt to speed up the process, some of these dairy companies take and deliver the milk samples for the farmer when they pick up his milk and then deliver the samples to a lab for testing. However, very often the companies put all the sample bottles together and submit them under the company name, making any trace back virtually impossible. Also, some farmers have 2 milking units on separate farms but they supply only one MRT result.

Another aspect of concern is where the milk is sent for the MRT. Are the labs where the milk is going accredited and do they have a certificate of competency to carry out the MRT? Our recent experience would suggest that this is an area where a lot of work needs to be done to ensure that no importer can question the validity of the tests on which SV's are basing their certification.

c) TB Testing

From the maps it is evident that Private Veterinarians must do a lot of the TB testing on these dairy farms. The results of these tests should be forwarded to the relevant State Veterinarian who then issues a TB Certificate based on the results presented to him. The first problem is of course that the certifying Veterinarian is a step removed from the testing and has to rely on somebody outside his control for interpretation of the test. The SV could in fact be two steps away if e.g. an Animal Health Technician carries out the test under the auspices of the private veterinarian. The most worrying aspect of this situation is the apparent lax attitude of some private practices to the requirements for and complexities of TB testing. In addition, it would seem that at present there is no standardized "TB certificate" for SV's to issue and there are questions about whether private veterinarians are entitled to issue this certificate if they carried out the test. So here again, validity and verification of our certification is open to debate and, by implication, so is the disease free status of the product.

d) Transport across Provincial boundaries

When any produce moves across a boundary that separates systems of control, problems are bound to arise. In these instances, the strength of the systems in place, the co-operation between the systems, their validity and their verification become as important as the product itself. What happens then when dairy produce moves directly from a farm in KZN to e.g. a Gauteng dairy company? What systems are in place to ensure that the produce is "safe" in terms of CA and TB when it leaves the farm and who is responsible for that assurance and who monitors what? At the moment there would not seem to be a viable system in place which means that produce of dubious quality could be moved and sold outside KZN borders without being subject to any health status monitoring.

e) The Dairy Companies

This set of problems became apparent when a dairy company wanted to export produce. Their first problem was that the requirements they had to meet differed depending on who they approached. For example, having approached the Department of Health they were given a set of requirements to meet. Having met these requirements they were understandably upset when VPH walked in and gave them a whole separate set of requirements to meet if they wanted to export. These requirements included the CA and TB certification of milk traceable back to the individual farms that were contributing to the export consignment. Under the current set up, this proved very challenging and in some cases impossible.

DISCUSSION

In discussion with one of the major dairy companies, the Department of Health, Veterinary Public Health and SV's, we came to the conclusion that we had to make a start by getting a database together that all role players would contribute to, have access to and which encompassed factors under our collective control. This database (as shown below) is divided into District Councils (as are our SV's) listing each known dairy farm in that area, together with where their milk goes to, the dates of their last Department of Health Inspection, the date of their last TB test, and listing their MRT results every month.

Business	Farm Name	Buyer	Lat	Long	Parlour No	Address	Phone No.	District	Last Health	Last TB	MRT-J

The hoped for outcomes of this exercise are as follows:

1) Each State Vet will be more acutely aware of the dairy farms in his area being served by private veterinarians and will have on record each farms current TB/CA status even if the private veterinarian comes from outside the DC.
2) Farmers not on this database but known to be producing dairy products can be identified and followed up.
3) Farmers that change the dairy company they supply or stop supplying known companies can be identified and followed up.
4) Dairy Companies will ensure that MRT's are submitted under each farms name.
5) The Department of Health will know which farms it should visit and when.
6) State Veterinarians and Private Veterinarians will co-operate more closely.
7) It will raise the level of awareness of the CA/TB issue in the minds of everyone involved.
8) There will be a record to consult whenever questions of certification and origin arise.

An added measure that will be undertaken is that each Veterinary practice involved in TB testing will be given the new TB manual as soon as it becomes available.

Obviously these measures alone cannot address all the issues raised but it is a practical start to addressing some of the problems and will, we hope, give impetus to solving the problems outside our span of control.

REACHING THE SMALL-SCALE DAIRY FARMER: RESULTS AND RECOMMENDATIONS AFTER GIVING COURSES IN COW HEALTH

P.S.A. Woods[1]

ABSTRACT

Access for poor, remote farmers to veterinary assistance is difficult and expensive, usually involving long time delays. In Southern Africa, subsistence farmers are developing to include small-scale commercial dairy enterprises. However, in some regions, government-provided veterinary services are limited by lack of funding, and diseases are spreading. Thus these small-scale farmers need to have appropriate information so they can recognize livestock problems and start treatment or preventive measures within their community. Because of time and distance constraints, together with disease epidemiology, this information is most effective if given to all surrounding farmers. Veterinarians can assist by identifying those prevalent disease conditions and management practices on the dairy farms that could be improved, and then providing focused and appropriate information to groups of farmers. Accordingly, in order to address large numbers of farmers, a project to hold day-long training courses through the network of local Dairy Cooperative Centres (DCC) in 10 regions throughout Zimbabwe was implemented. Then the impact of short courses about on small-scale dairy farmers' knowledge and behaviour was assessed.

This project comprised 3 phases:
1) Initial, baseline on-farm interviews of farmers (n=141) about dairy management and health problems.
2) Open invitation to a one-day course at their local DCC, leading to an unprecedented attendance of over 600 farmers (40% women).
3) 3 months post-course, re-interviews about knowledge from the course and changes instituted.

Attendance resulted in improved milking hygiene practiced by nearly 100% of farmers (previously 81%). Identification of mastitis cases increased (90% of herds compared 61% before). A disturbing note was the lack of knowledge about the importance of Brucella vaccinations, combined with not always pasteurizing milk. However, the active diffusion of information after the courses was encouraging, every attendee having told at least one person about what they had learned. This teaching role of veterinarians could be the first step in establishing partnerships for community-based herd-health programmes

INTRODUCTION

Access for poor, remote farmers to veterinary assistance is difficult and expensive, usually involving long time delays. In Southern Africa, some farmers are developing from subsistence activities to small-scale commercial dairy enterprises. However, government -provided veterinary services are limited by lack of funding, and diseases are spreading. Thus these small-scale farmers need to have appropriate information so that they can recognize livestock problems and start appropriate treatment or preventive measures. Because of time and distance constraints, together with disease epidemiology, this information is most effective if given to all surrounding farmers.

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Accordingly, in order to address large numbers of farmers, a project to hold training courses through local Dairy Centres (DC) in 10 regions throughout Zimbabwe was implemented.

MATERIALS AND METHODS

This project comprised 3 phases:

Phase 1) Initial, baseline on-farm surveys of farmers (n=141) about dairy management and health problems. Interviews were structured, and included some open-ended questioning to obtain additional opinions. Farmers were not identified by name on questionnaires, and could voice their views in a respectful, safe, and private environment.

In addition, to see how the community was already dealing with animal health problems, questions were included about the existence of "Local Livestock Experts" (LLEs)or those locals who were considered knowledgeable by their community and who people would ask for help or advice. These LLEs could be precursors of an informal network of Community Animal Health workers.

Phase 2) Open invitation for all interested farmers to a one-day course held at their local DC, leading to an unprecedented total attendance of over 600 farmers (40% women).
The objectives of the course were to:

- Recognise certain livestock diseases that are straight forward to diagnose and treat
- Suggest appropriate treatments, dosage and also emphasise nursing methods to improve the likelihood of success
- Advise preventive measures
- Teach basic record-keeping system

Phase 3) 3 months post-course, re-interview the same farmers as in Phase 1 about their attendance, any knowledge from the course and changes instituted, together with their suggestions to facilitate learning.

RESULTS

During the Phase 1 baseline survey, 65% of households reported that the head of household (HH) decided the dairy herd management, and 31% of farms reported the HH wife in charge. Male employees (23%) and male children (19%) were often involved in the milking (HH 36% and HH wife 22%). It is important to include in the courses both the decision makers, (who decide the allocation of finances), as well as the milkers who have to recognise disease.

For the Phase 3 survey, paired interviews were obtained from farms in 8 DC regions (n=105). 97% of farms had heard about the course and 93% had at least one household member attend. 97% of attendees reported that the course had been useful, and instituted some of the knowledge learned (Table 1). There was also an active exchange of information after the courses, every attendee having told at least one person about what they had learned.

82% of farmers reported having had a sick cow in last 24 months before the course, (70% after the course), and the percentage of farmers who reported their sources of help for the last sick cow is shown in Figure 1.

Table 1. Comparison of pre- and post-course implementation of recommended management procedures that the farmers reported to perform

Procedure	Percent of farmers performing before the course (n=141)	Percent of farmers performing after the course (n=105)
Use of milking salve	93% do, 3% use Vaseline, 2% use milk from bucket	99% use milking salve every time they milk
Teat dipping	81% farms do TD at least once daily	86% do teat dipping every time
Use of Dry Cow therapy	67% farms had heard of this, but only 24% always used DCT	90% heard, 67% always used, and 22% sometimes
Mastitis problem	61% farms recognised mastitis	90% recognised mastitis
Treatment of mastitis	16% used no treatment, 80% used teat dip or other mammary treatment, but only 10% used IM antibiotics	67% used intramammary antibiotics, only 1% no treatment
Use of acaricide	52% spray cattle, 16% dip,32% both	81% spray cattle, rest dip
Vaccinate cattle	80% vaccinate, mainly QE or Anthrax	No change
Vaccinate against Brucella	NONE, no vaccinations were performed	76% know that it is a legal requirement[1]

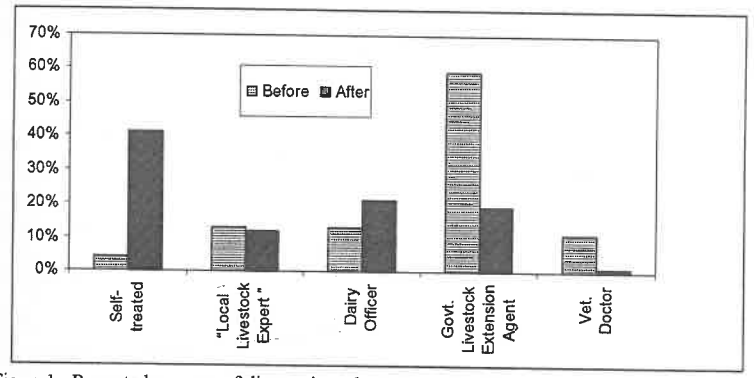

Figure1. Reported sources of diagnosis and treatment for last sick cow pre- and post-course

DISCUSSION

The percentage of farmers who themselves were able to identify and treat various cattle diseases increased from 4% to 41% after attending the course. The use of a local livestock expert (LLE)

[1] Subsequent to the course, at at least one DCC, the farmers vaccinated all their cattle (including adults) with S19 vaccine resulting in false sero-positives in a later serosurvey.

remained constant and important. Most farmers (75%) knew of, and had used the services of an LLE. Most LLEs (30%) had gained their expertise after attending agricultural courses, and 68% were male. In 30% of cases, the farmers paid the LLEs for their assistance, which was the same percent as for more qualified persons such as dairy officers or extension agents. LLEs remained an important source of assistance even after the course.

This research could not have succeeded as well as it did without us deliberately employing communication skills. Team members were sensitized to the importance of listening to what the farmer was saying, to then reflect one's understanding, and invite further input.

Holding interviews on-farm prior to developing the courses was a useful step, and I feel that facilitating the farmers meeting the veterinarians personally greatly increased attendance at the courses, especially amongst farmers in remote locations. Farmers could also voice their concerns in a safe, confidential environment on their own farms, and being on the farm allowed us to interact with all the family members working with cattle, leading to more caretakers attending the courses, and possibly increasing the impact of the courses. Another aspect of making the courses accessible to all household members included holding the courses at convenient times & locations.

These phase 1 survey visits established rapport and professional trust, and allowed the veterinarians to see on-farm conditions, and identify potential problems to include. Courses were developed in response to expressed needs. A deliberate effort made to avoid adopting a "top-down" or "I know what is best for you as I am a veterinarian" approach. The course materials were relevant to the farmers' concerns and included diseases grouped together with nursing, treatment and prevention methods. We found pictorial flow charts to be effective teaching aids, and that question times allowed discussions.

CONCLUSIONS

- Farmers were experiencing problems as the Government -provided veterinary services are now limited by lack of funding, and diseases are spreading. Many farmers have to cope with these problems without veterinary assistance. Therefore they want knowledge to enable them to make informed decisions about management techniques that could prevent livestock disease, treat livestock disease, increase productivity, and decrease wastage.
- The courses were extremely well attended, and more were requested.
- Active diffusion & utilisation of information after the courses was encouraging, every attendee having told at least one person about what they had learned.
- The role of informal "local livestock experts" in providing animal health care is important, especially in more remote areas.
- Prevention of zoonosis such as Brucellosis, especially where natural fermentation of milk is used without pasteurization should be a priority.
- Private pharmaceutical companies can be a valuable partner in providing knowledge on animal health care in small-scale areas.
- Group communication is an essential, effective tool for veterinarians working in developing countries with the rural poor. However, communicating with groups can be a problem for veterinarians, especially those from developing countries who are not trained in communication skills. These skills are essential tools for all veterinarians to be able to use & incorporate into whatever and where-ever their field

ACKNOWLEDGEMENTS

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CYSTICERCUS BOVIS IN NORTHERN NAMIBIA

L. Shikongo[1] & C.M.E. McCrindle[1]

SUMMARY

There are significant levels of cysticercosis bovis in cattle in North Central Namibia. Most rural communities in the north are resource poor and have little access to information and measures against tapeworm infestation. This paper discusses the demographics of the population and the postulated prevalence of the disease as well as its impacts on the population of North Central Namibia. Between 60-70% of Namibia's population practice subsistence agro-pastoralism on communal land that constitutes 41% of total land area (Vision 2030, 2004). Livestock, including cattle, sheep, goats, swine, donkeys, and poultry, is used for alimony, and as such plays an important part in the society. In addition, meat and milk are important dietary components for the majority of the population. North-central region has 780 149 inhabitants comprising of 54, 3 % women and 45, 7% men, which corresponds to 42, 6 % of the total Namibian population of 1 830 330 (National Planning Commission (NPC), 2003). Almost half of the entire population live here on just 6% of the Namibian territory. The inhabitants of these regions are mostly resource-poor subsistence farmers and grow mainly vegetables, millet and maize while keeping cattle and goats. Ovambo ethnicity prevails in the region, which is subdivided into seven groups: Ndonga, Ngandjera, Mbalantu, Kwanyama, Kwambi Kwaludhi, and Kolonkadhi. Abattoir records showed a postulated prevalence of between 7.6 and 9 % in slaughtered cattle from this area. However these are only those that have followed the formal marketing chain. Circa 40,000 of the 580,000 in the area followed the formal path over a course of 5 years. It is estimated that these cattle are only about 25% of the cattle consumed. The area in question is rural, highly populated and lacking in infrastructure and basic sanitation. It was therefore concluded that cysticercosis poses a significant zoonotic risk to the human population.

INTRODUCTION

Parasitic zoonoses are those parasites that are transmitted between vertebrate animals and man (Carlos, Armando & William, 2003). Taeniasis/Cysticercosis is a tapeworm-related parasitic zoonoses, occurring in human small intestines at the adult stage, whereas the larval stage occurs in cattle muscles, causing bovine cysticercosis (Wanzala, Onyango-Abuje, Kang'ethe, Ochanda & Harrison, 2002). Bovine cysticercosis is a muscular infection of cattle caused by the larvae of the human intestinal cestode *Taenia saginata* (Onyango-Abuje, Nginyi, Rugutt, Wright, Lumumba, Hughes & Harrison, 1996). The agent is recycled back to people when they ingest meat from cattle, which have become infected when they ingested infested human feces (Waltner-Toews, 2004).

The economic significance of this parasite may be considerable, due to downgrading and condemnation of carcasses (Dorny, Phiri, Gabriel, Speybroeck, & Vercruysse, 2002) and it is cosmopolitan in its distribution (Dorny, Vercammen, Brandt, Vansteenkiste, Berkvens & Geerts, 2000; Dorny *et al.*, 2002). It is estimated that the prevalence of *T.saginata* varies between 1-10% worldwide (Onyango- Abuje *et al.*, 1996).

Tapeworms in man and "measles" or cysticercosis in cattle are distributed worldwide, but occur more particularly in underdeveloped countries (du Preez, 1997) moreover in rural areas in the developing countries of Africa (Dorny *et al*, 2000) and South Africa (Giesecke, 1997), where access to safe water and basic sanitations are limited. The incidence of infestation varies according to the

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geographical distribution of animals and man, the level of socio-economic development and standard of meat hygiene control.

According to Hunter (1994), a number of measures are employed in the control of cysticercosis. Firstly, during meat inspection, strict measures are taken at abattoirs to screen for cysticercus larvae in beef. Meat inspection, which is the most important public health control measure, identifies only a minor fraction of heavily infected animals, and also only when it is too late to avoid losses (Wanzala *et al.*, 2002). Hunter (1994) described food treatment as the second method. Meat, which is lightly infested with measles, may be made safe by cooking at 100°C for 2.5 hours. Also freezing at -10°C for 10 days or pickling at -10°C for 21 days is recommended for meat treatment measures (du Preez, 1997). Thirdly, the regular deworming of people will prevent the excretion of eggs and so prevent infestation of cattle (Hunter, 1994).

There are significant levels of *cysticercosis bovis* in cattle in North Central Namibia. Most rural communities in the north are resource poor and have little access to information and measures against tapeworm infestation. This paper discusses the demographics of the population and the postulated prevalence of the disease as well as its impacts on the population of North Central Namibia.

MATERIALS AND METHODS

A literature study was undertaken on the human, livestock and farming systems of North Central Namibia. The findings were used to discuss the reasons for the presence of cysticercosis, the zoonotic potential and ways in which the disease could be controlled

RESULTS

Between 60-70% of Namibia's population practice subsistence agro-pastoralism on communal land that constitutes 41% of total land area (Vision 2030, 2004). Livestock, including cattle, sheep, goats, swine, donkeys, and poultry, is used for alimony, and as such plays an important part in the society. In addition, meat and milk are important dietary components for the majority of the population. Extension services were non-existent in many parts prior to independence and they remain weak. Accesses to agricultural advice, credit, new technologies and markets were virtually non-existent. The outcome of this has been the prevalence of underdeveloped and inappropriate farming systems with a progressive decline in output. The modes of extension in Namibia used by the Directorate of Veterinary Services (DVS) in animal health-oriented extension shortly after independence were through farmers' days, farmer training courses, study groups, occasional lectures and pamphlet distribution (Schmidt-Dumont, 1994). Over the production period of 2000 to 2004, 3232 measles detection were recorded from the 40 373 cattle slaughtered at Meatco Oshakati Abattoir (Table 1).All organs and carcass portions should be kept together and correlated for inspection before they are removed from the slaughter floor.

The study area is located in the northernmost parts of the north-central region of Namibia. North-central Namibia, formerly known as Ovamboland, comprises of Omusati, Ohangwena, Oshana and Oshikoto regions and is nowadays known as the "Four-O Regions". It borders on the Kunene region to the west, on the Okavango region to the east, on the Etosha National Game Park and on the commercial farms of Tsumeb to the south, and on Angola to the north (Franco-Namibia Rural Development Project (FNRDP), 1993). Major town centres include Tsumeb in Oshikoto, Ondangwa, Oshakati and Ongwediva in Oshana, Outapi in Omusati, and Oshikango and Ohangwena in Ohangwena regions.

The area is semi-arid with extremely variable and unreliable precipitation (Department of Fisheries and Water (DFW), 1990) with an average annual rainfall of 200 to 500 mm (FNRDP, 1993). Seasonally, the area receives flooding water down an inland delta of drainage channels that reaches from the north to the Etosha Pan, which are linked to the Cuvelai river system in Angola.

Table 1. Prevalence of cysticercosis bovis at Oshakati Abattoir

Year	Cattle slaughtered per year	Measly detentions per year	Prevalence (%) of measly slaughtered cattle
2000	12 204	973	8, 0
2001	7 888	713	9, 0
2002	10 561	798	7, 6
2003	4 411	347	7, 9
2004	5 309	401	7, 6

The countryside and landscape is generally flat, monotonous and dotted with traditional settlements. Mopane, Marula and Wild Fig trees turn up occasionally between the fields and the kraals, and one can also find Makalani palm trees. Found within this plain are gentle undulations with local depressions known as oshanas. Pre-existing information regarding the prevalence of disease is shown in Table 1.

According to the 2001 Population and Housing Census (Table 2), the North-central region has 780 149 inhabitants comprising of 54, 3 % women and 45, 7% men, which corresponds to 42, 6 % of the total Namibian population of 1 830 330 (National Planning Commission (NPC), 2003). Almost half of the entire population live here on just 6% of the Namibian territory. The inhabitants of these regions are mostly resource-poor subsistence farmers and grow mainly vegetables, millet and maize while keeping cattle and goats. Ovambo ethnicity prevails in the region, which is subdivided into seven groups: Ndonga, Ngandjera, Mbalantu, Kwanyama, Kwambi Kwaludhi, and Kolonkadhi (FNRDP, 1993).

Table 2. Estimated no of people in each of the four political North-central regions in 2001 (NPC, 2003)

Region	Total Population
Ohangwena	228 384
Omusati	228 842
Oshana	161 916
Oshikoto	161 007
Total	780 149

Livestock

Agriculture in Northern Namibia is entirely extensive and based on natural pasture and indigenous breeds of livestock. According to the DVS national census of year 2002, cattle and goats are the dominant livestock species in the communal areas, constituting 66% (1.6 million) and 73% (1.4 million) respectively of Namibia's total population of these animals. Sheep are relatively scarce (350 000) constituting only 15% of the national flock. The rest of the domestic species in the communal areas are of low economic importance, because of their paucity and/or their low per unit economic vale. These include indigenous free ranging poultry estimated at 507 017, donkeys (288 219), horses (14 322) and pigs (40 688) (NASSP, 2003).

Table 3. Estimated population of cattle (Mendelson, Obeid and Roberts; 2000)

Region	No of cattle
Ohangwena	130 000
Omusati	218 000
Oshana	39 000
Oshikoto	193 000
Total	580 000

Farming Systems

The Namibian agricultural sector is divided into a communal farming sub-sector, where farmers operate on land operated under a communal tenure system, and a commercial farming sub-sector where farmers operate on freehold title deed land (National Agricultural Policy, 1995). Agriculture in the NCAs consists of a mainly non-income generating production system, and very limited cash exchange of local produce. Farmers in these areas are mainly engaged in subsistence rainfed cropping and extensive livestock production, characterised by low levels of productivity, high variability of output from one year to the next and a high degree of poverty, household food-insecurity and malnutrition (National Agricultural Policy, 1995). Much of the agricultural work at household level is customarily handled by women. For communal farmers within north central Namibia, livestock represent the means by which households survive drought periods through slaughter and/or sale of animals (Africa Institutional Management Services, 2002).

DISCUSSION

Abattoir records showed a postulated prevalence of between 7.6 and 9 % in slaughtered cattle from North Central Namibia. However these are only those that have followed the formal marketing chain. Circa 40,000 of the 580,000 in the area followed the formal path over a course of 5 years. If one estimates an offtake of 20% per annum, the amount that are consumed or slaughtered would be 116000 per annum or 580,000 over 5 years. At an estimated prevalence of 8 %, 9280 cattle would be positive for cysticercosis. In rural areas, cattle are informally slaughtered and no meat inspection is done. The meat may also not always be properly cooked as population in that area usually barbeque the meat over wood fires. The demographic profile also follows that of high risk communities as described earlier (du Preez, 1997; Dorny *et al*, 2000 and Giesecke 1997), *viz* rural communities with limited infrastructure and lack of basic sanitation and access to clean water. It is thus probable that there is a significant zoonotic risk in these areas. It is also concluded that the low proportion od animals following the formal route may be linked to knowledge that animals slaughtered at the abattoir can be condemned and the owners lose their investment.

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A DYNAMIC MODEL OF THE COST OF BRUCELLOSIS AND A COST-EFFECTIVE CONTROL OPTION IN A FIFTY-SOW PIGGERY IN NIGERIA

I. Ajogi[1]

SUMMARY

A dynamic model of the cost of Brucellosis in a fifty-sow piggery was built and the benefit-cost analysis conducted. The feasibility of the two control options, namely 1) testing and depopulation with replacement, and 2) vaccination of females with possible abortions in case of vaccine failure, were compared in order to determine the best choice. The model showed that the herd "without" Brucellosis was valued at US $157,528.80. The same herd "with" Brucellosis has a reduced value U$$155,402.7. Effect of Brucellosis on the herd is 1.35%. The benefit-cost ratio of vaccinating the herd is 17.6 while that of testing, depopulation and replacement is 11.4. The former would appear to be a better choice of control.

INTRODUCTION

Early incidence of swine Brucellosis in Nigeria was reported by Mettam (1947). Later Bale and Nuru (1985) sampling 443 pigs from 6 piggeries found that 12 (2.7%) of them had Brucella antibodies. *Brucella suis* was isolated from two of the pigs. Ajogi (2003) presented an economic model of establishing a fifty-sow piggery in Benue State of Nigeria and found it viable and cost-effective.

The effect and control of diseases of economic importance such as Brucellosis on the herd was however not addressed. The current study presents a model of the effect and the feasible control option of Brucellosis in the piggery.

MATERIALS AND METHODS

The basic information about the herd structure and litter size of the piggery unit have earlier been provided (Ajogi, 2003).

Initial (foundation) breeding stock comprised 50 sows and 7 boars. There were two litters of piglets per annum.

A total of 827weaners are usually reared to finishers weighing 81kg.

1. Annual value of stock.

This comprises annual value of finishers and value of initial stock. Market value of a kilogram of pork is US$ 2.2 (1KG = US$ 2.2 where US$1 = ₦140, Nigeria currency).

Value of finishers and initial stock = 884x81kgxUS$ 2.2= US$157,528.8.

2. The effect of the disease

This is calculated using the formula: $A_0 = A \pm Er$ (Putt et al. 1987), where A_0 = value of stock "without" disease, A = value of stock "with" disease, E = disease effect on parameters and r = incidence of disease.

Incidence of swine Brucellosis in Nigeria is 2.7% (Bale and Nuru, 1985).

Abortion rate due to Brucellosis is 50% (assumed).

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3. Test, Depopulate, Replacement and vaccination costs.

Incidence rate = 2.7%

Infected animals are sold at US$133.70 each.

Depopulated animals are replaced at US$213.80 each

Cost of blood sampling and laboratory testing = US$2.50

Cost of labour and vaccine = US$3.00

4. Control Options

Two control options, namely:

Test, depopulated, replacement

Vaccination of female pigs with risk of abortion in case of vaccine failure

were compared using the benefit – cost ratio method as described by Ellis and James (1979a, 1979b). The better alternative, which also has a higher benefit – cost ratio, was chosen.

RESULTS

Value of stock "without" Brucellosis in a year was obtained by:

(827+57) × 81kg × US$2.20/kg = US$157,528.80

Value of stock "with" Brucellosis is obtained by substituting in the equation

A_0 = A + Er

= US$157,528.8 - US$157,528.80 × 0.027

= US$155,402.20

From the calculations above, Brucellosis appear to have reduced the value of the herd by 1.35%.

Cost of testing, depopulation and replacement:

Number of pigs tested = 884

Cost of testing at US$2.50 per pig twice a year = US$2.50 × 884 × 2 = US$4,420.00

The proportion of pigs infected = 2.7% × 884 = 23.9 ≈ 24 pigs.

About 24 pigs are depopulated yearly at the rate of US$133.60

Cost of depopulating 24 pigs = 24 × US$133.60 = US$3,206.40

The cost of replacement of 24 pigs = 24 × US$213.60 = US$5,126.40

Total cost of testing, depopulating and replacement = US $ 12,752.80

Cost of vaccination with abortion if there is failure:

There are 469 females to be vaccinated and 50% of which are at risk of abortion.

Unit cost of labour and vaccine = US$3.00

Cost of vaccinating 469 pigs = 469 × US $ 3.00 = US$1,407.00

Cost of foetal losses in case of abortions:

Value of piglet at birth = US$30.00

Cost of foetal waste = 469 × 0.5 × US$30.00 = US$7,035.00

Total cost of vaccination with occasional vaccine failure leading to abortion:

= US$7,035 + US$1,407 = US$8,442

The benefit of any of the programmes is obtained by subtracting the cost of that programmme from the value of the herd "without" Brucellosis.

The benefit of testing, depopulation and replacement

= US$157,528.80 – US$12,752.80 = US$144,776.00

The benefit of vaccination and possible cost of abortion

= US$157,528.80 – US$8,442.00 = US$149,081.80

The benefit – cost ratio of the two programmes:

The benefits and cost are discounted at a 10% discount rate

The formula $\frac{\text{discounted benefits}}{\text{discounted costs}}$ is used.

Testing, depopulation and replacement = $\frac{131,614.50}{11,593.50} = 11.4$

Vaccination with abortions = $\frac{135,528.90}{7,674.50} = 17.6$

DISCUSSION AND CONCLUSION

Much work is yet to be done on swine Brucellosis in Nigeria. The little available information on the subject has been used in modeling the situation in just one of the many of such farms in Nigeria. If the result of this work were magnified to cover the whole country, the cost of swine Brucellosis will be enormous.

Only few farmers would see reason for selling the culled pigs at lower prices. There would therefore be a problem of convincing farmers to depopulate the infected pigs. It is possible, out of ignorance, the unsuspecting consumer of pork and sausages are being infected.

Vaccination of female pigs is a better choice in the control of swine Brucellosis on the farm. Allowance is however given, in case of vaccine failure, for the cost of resultant abortion. The value of piglet used here is modest and could be higher. That being the case, vaccination may not be the better choice of control. Pigs are not routinely vaccinated against Brucellosis in Nigeria.

Depopulation of infected pigs is the usual choice of control in piggeries (Seifert, 1996). Our finding however proves otherwise. We believe with a careful use of this method it will still be a better choice in future when many farmers would appreciate the implications of keeping Brucella infected animals in their herds.

CONCLUSIONS

1. Annual value of herd "without" Brucellosis = US$57,28.80
2. Annual value of herd "with" Brucellosis plus resultant abortion (abortion rate 50%) = US$155,404.20
3. Annual cost of test, depopulated with replacement =US$144,776.00
4. Annual cost of vaccination and possible vaccination failure (resultant abortion) = US$149,081.80
5. Benefit – cost ratio of test, depopulate with replacement = 11.4
6. Benefit – cost ratio of vaccination with abortions = 17.6
7. The model shows that vaccination appears to be the better choice of controlling swine Brucellosis in the chosen piggery since the benefit – cost ratio of 17.6 is higher than that of 11.4 obtained for test and depopulate with replacement programme.

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THE APPLICATION OF ACTIVE SURVEILLANCE AND RISK ASSESSMENT PRINCIPLES IN THE CONTROL OF BOVINE MASTITIS

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SUMMARY

No doubt mastitis is one of the biggest enemies of dairy cows with big potential to destroy cows' udders and disturb milk production, but it can also be prevented, cured and even eliminated from herds. However, to be able to do so, a finely integrated approach is needed. Early and accurate clinical and laboratory diagnosis, adequate treatment and good herd management practice together constitute some of the key elements for consideration in such an approach. The author discusses a set of epidemiological approaches with particular reference to active disease surveillance and risk analysis principles that may be applied strategically in response to the challenging offense posed by bovine mastitis. The paper provides a practical demonstration of the way these principles can be implemented on terrain, the expected outcomes of a successful application and the major challenges encountered.

INTRODUCTION

Surveillance can be defined as the process of systematic collection, collation, and data analysis. Thus, animal disease surveillance implies an active system, where directed actions are taken if the data indicate that disease prevalence or incidence exceeds a predetermined threshold. A well-functioning disease surveillance system provides information for planning, implementation, monitoring, and evaluation of control and eradication programmes (McCluskey 2004). Surveillance activities can be divided into two major categories: scanning and targeted surveillance.

Scanning surveillance accesses readily available populations and thus available biological samples to estimate the extent of disease in that population or as a case finding mechanism. Targeted surveillance specifically identifies groups or subpopulations of animals with a high projected risk of acquiring or disseminating disease. These populations are then sampled at a higher rate than populations considered at lower risk of disease (McCluskey 2004).

In contrast, disease monitoring describes ongoing efforts at assessing the health status of specific animal populations. However, the line between disease monitoring and disease surveillance is neither very wide nor straight, which is why the two terms surveillance and monitoring are often used interchangeably in animal health programmes (Salman 2003).

Risk analysis on the other hand is a tool intended to provide decision-makers with an objective, repeatable and documented assessment of the risks posed by a particular course of action (MacDiarmid 1997). Ideally, it provides the basis for anticipating, amongst other, what can possibly go wrong and the likelihood of it to go wrong. This obviously, opens ways to proper planning and management of what can be done to reduce either the likelihood or the consequences of it going wrong (MacDiarmid & Pharo 2003).

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RELEVANCE OF SURVEILLANCE AND RISK ASSESSMENT PRINCIPLES FOR THE CONTROL OF BOVINE MASTITIS

The programmes for the control of bovine mastitis should be designed to run as risk management projects with key components including risk assessment, risk communication and risk management.

Risk assessment (RA) principles

This component should be designed to identify mastitis causing and contributing factors, such as bacteriological, cytological, mechanical, environmental, etc. and to determine the prevalence and incidence of mastitis in the herd. The tools to do this are laboratory investigations of specimens, clinical examinations of cows/udders, inspection and evaluation of the milking machines (Table 1) and the environment.

Bacteriological and cytological hazard identification: this is achieved through laboratory investigation of specimens. The processes include:

(a) The count of somatic cells in milk samples (SCC) (preferably quarter milk) using appropriate technological device such as the Fossomatic machine or coulter counter.
(b) Bacterial culture, identification and antimicrobial sensitivity testing using validated standard microbiological procedures;
(c) Total bacterial count on bulk milk using standard microbiological procedures or available rapid commercial techniques such as the Petri film technique;
(d) Coliforms and *E. coli* enumerations also using the Petri film technique;
(e) Total plate count, and Coliforms & *E. coli* counts on water using validated standard microbiological procedures or rapid commercial techniques such as the Colilerts technique.

Hazard identification in the environment: Milking machine

Table 1*. Example of elements to be considered when evaluating the efficiency of the milking machine

	Item	What to check
Checks and tests done prior to milking	V-belt	Tension, alignment of pulleys, oil level, presence or not of complete safety cover
	Vacuum reserve tank	Hygiene, condition, float valve
	Layout of the system	Number of bends, flow restrictions, slope of milk lines, length of long milk tubes, volume of cluster, height of flow meter in relation to udder height
	Vacuum meter reading	Whether it is working or not
	Sizing of lines	Milk line diameter, wash line diameter, vacuum line diameter
	Vacuum control valve	Type, mounting position, condition
	Condition of rubber parts	Long milk tubes, long vacuum tubes, short vacuum tubes, couplings
	Teat liners	Liner fit, barrel diameter, line alignment, liner tension, inner surface, date of last replacement

	Item	What to check
Checks done on the milking machine when running but not working:	Accuracy of the vacuum gauge	
	Pulsation rate	Pulsation rate at teat liner Pulsation variation between units
	Teat liner collapse	
Checks and tests done on the milking machine while working	System vacuum reserve	Reading at end unit, reading at vacuum control valve, deviation in vacuum gauge maximum workload, whether air is sucked at control valve
	Vacuum stability at teat end	Low producers, mild producers, high producers
	Stimulation	Duration, stimulation till attachment
	Milking speed	
	Milking time	
	Frequency of liner slipping	
	Residual milk	
	Teat condition post milking	Colour, degree of swelling, teat end condition

*Adapted from Petzer 2003

Hazard identification in the environment: Cleaning process

Hygiene examination of milking equipment (liners, clusters, bottles, lines, dead ends, end unit, pulsators, vacuum control valve, vacuum reserve tank, utensils, floor, walls) and hygiene evaluation outside the parlour (waiting area, draining, etc) are important steps to be considered in identifying the causes and factors that contribute to the occurrence of mastitis (Giesecke et al. 1994).

Hazard identification in the host: Clinical examination of udders

Table 2*. Examples of elements to be considered during clinical examinations of udders as a process of hazard identification in the host

Cows ID	Quarters reference	Clinical evaluation indicators in cows' udders					Remarks
		Fibrosis	Atrophy	Nodules	Teat canal	Udder depth	
Cow 1	RF	(1-4)	(1-4)	(1-4)	(1-4)	(1-4)	
	RH	(1-4)	(1-4)	(1-4)	(1-4)	(1-4)	
	LF	(1-4)	(1-4)	(1-4)	(1-4)	(1-4)	
	LH	(1-4)	(1-4)	(1-4)	(1-4)	(1-4)	
Cow 2							
	RH	(1-4)	(1-4)	(1-4)	(1-4)	(1-4)	

*Adapted from Petzer 2004. 1 = slight; 2 = mild; 3 = severe; 4 = very severe

Risk communication (RC) principles

The RC component should provide communication pathways in the programme for mastitis control. Efficient means of communication (e.g. telephone, fax, mail, etc.) should be utilized to correctly pass the information to different users of the programme at different levels. The information about the hazardous and contributing factors extracted from laboratory and field data (Tables 1 & 2) should be fed back through the system by means of formal periodic reports. The frequency and quality of reporting should be determined and regularly monitored. Proper communication of information is key to determining the most adequate measures to be applied to reduce either the likelihood or the consequences of the herd udder health situation to go wrong or to worsen if it went already wrong following the RA findings.

Laboratory reports should contain data on bacteriology and SCC on individual cows or quarters, antimicrobial sensitivity testing on important pathogens, estimation of the loss incurring in milk production, current and serial herd udder health data, udder health statistics on mastitis cases (Figure 2), teat canal infections (Figure 3), non-specific disturbances (Figure 4), a herd percentage of each of the above diagnostic cases to determine herd udder health trends and patterns (Figure 1), which are prerequisite for inducing targeted corrective actions, and appropriate recommendations.

Field reports should include individual cows and udder health conditions, milking machines proficiency and hygiene conditions.

Table 3. Example of herd udder health statistical report after laboratory examination of quarter milk samples

Diagnostic cases	Quarter x Percentage values					Total
	Micro-organisms	Right front quarter	Right hind quarter	Left front quarter	Left hind quarter	
Mastitis	*Enterobacteria*	2.27	2.27	0.00	0.00	4.54
	S. aureus	4.55	0.00	2.27	0.00	6.82
	S. epidermidis	0.00	2.27	2.27	0.00	4.54
Teat infection	Contamination	0.00	4.55	2.27	6.82	6.82
	S. aureus	6.82	4.55	2.27	6.82	20.46
Non-specific diagnosis	-	2.27	4.55	4.55	11.36	22.73
Normal	-	9.09	6.81	11.37	6.82	34.9
	Total	25.00	25.00	25.00	25.00	100.00

Risk management (RM) principles

This component is about choosing and applying measures that are deemed fit to meet the appropriate level of controlling mastitis under conditions unique to each herd. Depending on circumstances, these measures may be preventive, curative or even destructive in order to restore the health in the cow population. At this stage what is wrong in the herd is presumably known. The selected measures of overcoming the situation will be more efficient when beside the knowledge of what is wrong; the likelihood of it to go wrong is also determined. Therefore, depending on circumstances, measures for restoring the health could include effective and efficient treatment regimen (for instance the approach when treating a dry cow and a lactating cow will differ), appropriate strategies for culling of cows and/or inactivation of udders, or a combination of one or more measures.

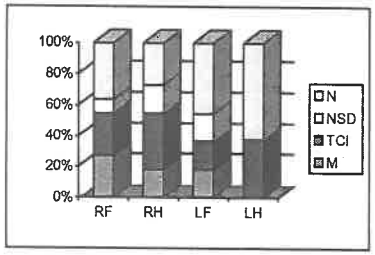

Figure 1: Graph showing overall picture of udder health conditions in the herd

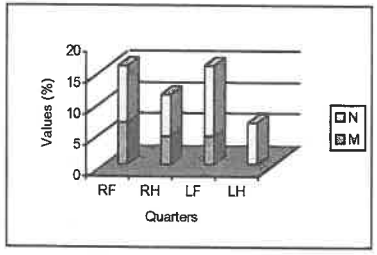

Figure 2: Graph comparing mastitis cases with normal quarters in the herd

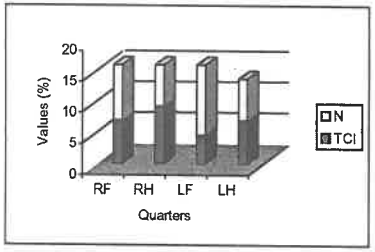

Figure 3: Graph comparing teat canal infection cases with normal quarters in the herd

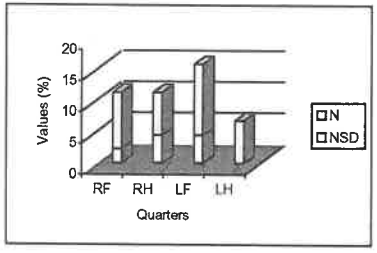

Figure 4: Graph comparing udder irritation cases with normal quarters in the herd

RELEVANCE OF QUALITY ASSURANCE (QA) FOR THE CONTROL OF BOVINE MASTITIS

Quality assurance is the activity of providing to all concerned the evidence needed to give confidence that the quality activity is being performed properly (ISO17025). QA should therefore be an integrated part of the design of any monitoring and surveillance system to ensure that it is capable of serving its purpose. Some important principles of QA such as "documentation and monitoring" should be equally applicable to surveillance and monitoring system (Salman 2003).

Like in any monitoring and surveillance system, documentation is a prerequisite for evaluating the quality of programmes for the control of bovine mastitis. All protocols on laboratory and field procedures should be documented in detail. This includes protocol for sample collection, handling and dispatching (quarter, composite and bulk milk, and water), processing and analysis of samples in the laboratory (SCC, bacterial culture & identification, and antimicrobial sensitivity testing), data recording and analysis, cows and udders examinations, milking machine inspections and hygiene monitoring.

The type of data to be collected in the laboratory and field (Tables 1 & 2) should be defined and their proposed uses in each of the steps of mastitis control should be known to all users preferably before the programme is implemented. The outputs expected from the programme depend entirely

on such data. Ideally surveillance and monitoring system for animal disease, should gather data on the agent (Table 3), host and environment (Tables 1 & 2) (Hueston 1993). This is why an effective mastitis control programme should be designed to include both the laboratory and field aspects as one comprehensive and integrated package.

Quality implementation can quickly become jeopardized if the steps taken at different levels of the programme are not standardized. These steps include the following items: interpretation of laboratory-generated results, and subsequent recommended actions to be taken on the field, interpretation of clinical diagnosis and subsequent actions to be taken. Similarly, training sessions should be organized for key laboratory and field role players to standardize important aspects of the programme such as aseptic collection and handling of samples, procedure for milking machine inspection, procedures for udders and teats examinations, and procedures for hygiene evaluation.

The entire programme should be subject to continuous evaluation to ensure that it continues serving its purpose. This can be achieved through the formation of a technical body or committee that is representative of all stakeholders. The mandate of such a body or committee should include the following:

(1) Monitor the smooth running of the programme in relation to the set objective
(2) Audit the laboratory QA system and GLP implementation in general
(3) Monitor adherence to set protocols and standards by all role players
(4) Assess the efficiency of implementation of control measures by customers
(5) Deal with complaints
(6) Assess the incidence of mastitis
(7) Detect causing and influencing factors of new mastitis cases in problem herds
(8) Discuss drugs supplies and bacterial resistance problems
(9) Review strategies in problem herds
(10) Discuss any relevant technical matter
(11) Formulate relevant recommendations
(12) Compile progress reports

CONCLUSION

Successful programmes for the control of bovine mastitis should be designed to run as risk management projects where active surveillance and risk assessment principles are applied. They should be comprehensive, integrated, broad-based and well coordinated. Since such programmes may generate huge amounts of data, systems for accurate data collection, collation, analysis and information management should be defined and put in place. The type of actions to be taken if the data dictate so should also be clearly indicated.

Despite its multiple facets and involvement of multiple expertise, the programme should still remain flexible and simple. Strict quality measures should be an important prerequisite to consider for smooth running of the programme. Such measures should be defined and observed to monitor the efficiency of each component of the programme. Lack of skills or appropriate technology may lead to failure of depicting real causes of mastitis or measuring their importance much as it can render the communication pathways very defective. As a consequence, wrong measures will be often taken making the risk management component a real failure.

Collaboration between experts from different fields such as laboratory diagnosticians, veterinary field clinicians and epidemiologists is required to generate reliable data and meaningful interpretation of such data before the release of adequate information that is needed for planning, implementation, monitoring and evaluation of control measures. These experts do not have to be located on one site. Distant means of communication can be utilized. The lack of epidemiologists, statisticians and mathematicians is by far the most experienced in the field of mastitis control. Consequently, the manipulation and interpretation of data are often carried out by average people, making the evaluation of possible risks and development of appropriate models for efficient control of bovine mastitis a difficult and almost impossible process.

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RISK MANAGEMENT IN A MOVEMENT OF CATTLE FROM THE FOOT-AND-MOUTH DISEASE BUFFER ZONE TO THE FREE ZONE, MPUMALANGA, SOUTH AFRICA

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ABSTRACT

The Ehlanzeni / Lowveld District of the Mpumalanga Province of South Africa borders onto the Kruger National Park (KNP), where Cape buffalo (*Syncerus caffer*) populations are persistently infected with foot-and-mouth disease (FMD) virus serotypes SAT-1, SAT-2 and SAT-3, bovine tuberculosis as well as theileriosis. Control measures such as movement control, vaccination and surveillance are enforced in farming areas adjacent to the KNP, in order to prevent transmission of the virus to susceptible animals.

Movement of cattle originating from the buffer zone, where FMD vaccination is routinely done, to the free zone is generally not allowed, but can be permitted according to the guidelines of the World Animal Health Organisation or Office International des Epizooties (OIE) after proving freedom from infection.

A motivated application to move an Afrikaner stud herd from the buffer zone in Mpumalanga to the free zone was received by the veterinary authorities. A protocol was drawn up to manage the risks involved in such a movement.

The protocol was strictly applied, but complications set in when unexpected positive serological results were obtained in cattle that formerly had tested negative. After infection was excluded as a possible cause of the serological reactions, other possibilities were investigated. A field trial was conducted in order to investigate one of the possible causes of positive test results, while some more trials are planned in order to facilitate future risk management in similar situations.

Another complication was encountered when bovine tuberculosis was diagnosed in the remaining part of the herd, while some cattle also showed serological reactions in respect of theileriosis. Eventually no disease outbreak was experienced in the free zone, while the establishment of the stud herd in the free zone was achieved.

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ECONOMIC OPPORTUNITY SURVEY OF SMALL SCALE DAIRY FARMERS IN CENTRAL NORTH WEST PROVINCE

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SUMMARY

The North West Province (NWP) of South Africa, particularly in the Central Region, has identified dairy farming as a priority as it has the potential, not only for job creation, but also as a sustainable source of high quality protein for rural communities. Purposive selection was used to identify 15 small-scale dairy farmers in Central North West Province and these were visited monthly. The research model was based on Action Research, that is the "plan-do-review" cycle with ongoing implementation and evaluation. The approach was holistic, considering not only the epidemiological triad (host-agent-environment) but also all extrinsic and intrinsic factors impacting on the small-scale dairy farming system under study. Participatory rural appraisal (PRA) revealed that none of the farmers had an adequate level of knowledge of animal diseases, stock remedies and vaccines. Two farmers used herbal medications. Fortunately, eight farmers mentioned that they contacted state veterinarians and animal health officers for assistance with animal health problems. None of them, however, made use of the private veterinarians who usually service commercial dairy enterprises in South Africa. Record keeping was poor or absent. Water quality was suboptimal with high levels of coliform bacteria. High bacterial and somatic cell counts in the milk were linked to Staphyloccocal mastitis which could pose a zoonotic risk as South Africa has a high level of immuno-compromised persons due to HIV infection. The probable reason for this is that many of the cows were purchased at stock auctions and may have been cull-cows. Bulk milk and quarter sampling is now being done regularly by the state veterinary services. These farmers are now enrolled in the North West Province mastitis programme and it is visualised that commitment of the state veterinary services to improving udder health and milk quality will have a positive impact on both human and dairy cow health .

INTRODUCTION

Southern Africa is characterised by a dual economy, where sophisticated commercial farming systems exist side by side with a range of traditional and small scale systems. Agricultural land is marginal and water is scarce, resulting in few areas suitable for dairy farming (Maree and Casey,1993). Since 1994, legislation has been promulgated to assist farmers to obtain land and resources for commercial farming (NDA,2005). Dairy farmers require a great deal of input from veterinarians in order to achieve internationally accepted standards for the production of safe milk and dairy products (NDA, 2005).

Available statistics show that there are approximately 257 000 dairy cattle in the NWP, with the greatest numbers in the Central Region (175 235) and smaller numbers in the Western (59 852) and Eastern (21 873) Regions. These cattle produce approximately 230.4 million litres of milk annually (12.5% of national production) with an estimated value of R304.1 million, excluding value-added products in the form of cheese, yoghurt, milk powder and others. These production figures originate from 45 714 cows in milk on a daily basis (17.8% of the dairy herd) that translates into an average production of 14 l /cow/day (Prozesky et al., 2003).

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 500 l /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. A further 18% of producers

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produce 500 to 1 000 l /day and another 18% between 1 000 and 2 000 l/day. The remaining 18% produce in excess of 2 000 l /day and can be truly regarded as large-scale, commercial ventures. Dairy farming, especially at the lower daily volumes (<500 l /day), is seldom the only enterprise and shares resource with other farming enterprises. The current trend in dairy farming internationally is away from smaller operations towards larger, specialized operations to increase profitability (NDA, 2005; Maree and Casey, 1993; Radostis, 2001). However, 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. In Kenya, it has been shown that suitable small-scale production systems, with the correct type of animal, can be feasible (Bebe et al., 2002). Whatever farming system is practiced, milk safety must of necessity be regarded as a priority.

MATERIALS AND METHODS

Purposive selection (Thrusfield, 1995) was used by the project leader, in collaboration with extension and veterinary services, to identify 20 previously disadvantaged dairy farmers, that could potentially be included in the study. In terms of the protocol, a production level of 500 l /day or less was considered to be a small-scale producer, irrespective of lard size or number of cattle. The Department of Agriculture, Conservation, Environment and Tourism (DACET) in the North West Province in collaboration with the Directorate of Land Affairs is placing potential dairy farmers on commercial farms bought for that purpose. It has recognised that there are deficiencies in the management of these dairy projects and they therefore deliberately selected farmers as representative examples of the problems faced by the group. The criteria for selection were willing participation; a genuine desire for assistance in improving their dairy farming skills and ownership of not less than five cattle whose milk is used for human consumption. After interviews, this number was decreased to 15 farmers so that an in depth study could be done and interventions monitored.

The research model was based on Action Research, that is the "plan-do-review" cycle with ongoing implementation and evaluation (McNiff, 1988; Waters-Adams & Nias, 2003). The approach was holistic, considering not only the epidemiological triad of host-agent-environment (Thrusfield, 1994) but also all extrinsic and intrinsic factors impacting on the small-scale dairy farming system under study.

After selection of the 15 farmers, a workshop was held with the farmers and their extension and animal health officers to discuss and prioritise short and long-term objectives using participatory methods (facilitated focus group discussions). A modified SWOT analysis (Strengths, Weaknesses, Opportunities and Threats), at the level of the farming families and the other stakeholders formed the first part of the PRA. A structured interview was developed from the findings of the workshop and background information gathered form DACET. This was followed by farm visits during which small-scale dairy farmers in the central region of North West Province were visited. A structured interview and 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. In the following year, the implementation of change was initiated using monthly farm visits and farmer's information days. Record keeping was instituted and milk and water sampling began. In the third year, udder health was instituted and general fertility and health of the cattle observed and recorded so as to develop further extension and training for both farmers and the extension and veterinary staff. Action research (plan-do-review) was ongoing throughout the period of the study.

RESULTS

Initial interviews were conducted with the people directly involved in managing the dairy cattle as part of participatory rural appraisal. A summary of the PRA findings is shown in Table 1.

Table 1. Summary of data obtained from the PRA done in 2002 and 2003

	Observation	Comments
General management	Background information from DACET	Obtained and recorded.
	Estimated milk production	Average milk production per cow =10.6 (range 3 to 25). Lactation length 124-300 days
	Water source	Mainly boreholes
	Milking management	Hand milking by 14 farmers. Cows milked once or twice a day. Calves sold or reared by cow.
	Milk sampling	Mastitis mainly caused by *S.aureus*. High bacterial cell counts
	Infrastructure checklist	Few bulk or cooler tanks
	Management	Three types of management: commercial, dual purpose or semi-commercial and traditional were found.
	Record keeping	Totally inadequate milk records, cow records, financial records
Diseases	State Veterinary Services	All farms were serviced by the State Veterinary Services, however state veterinarians were insufficient in some areas. Extension officers were present on all farms
	Farmers knowledge of dairy cattle diseases	Inadequate
	Use of stock remedies and vaccines	Lack of knowledge

The gender ownership distribution was 60% (n=9) male farmers, 13% (n=2) female farmers and 27% (n=4) community projects. The age distribution of farmers ranged from 21 to 80 years old (Fig 1). The educational levels are shown in Fig 2.

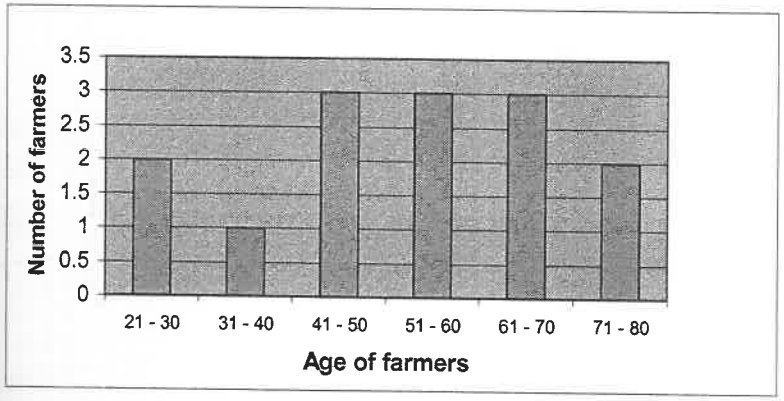

Fig 1. Age distribution of farmers

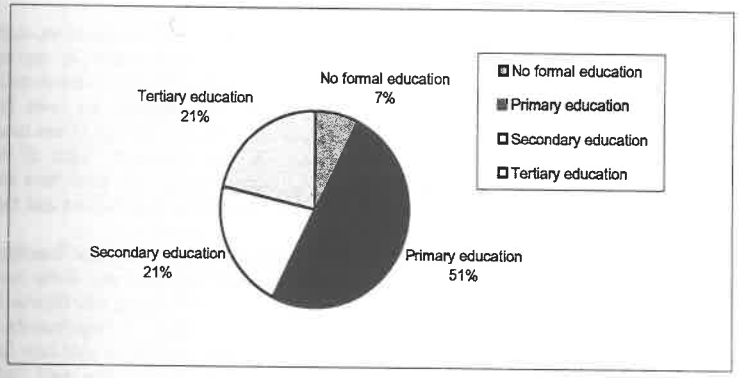

Fig 2. Education levels

Only one farmer was a member of the milk producer's organisation in South Africa (MPO). Most participants had less than 5 years experience of dairy farming (Fig 3).

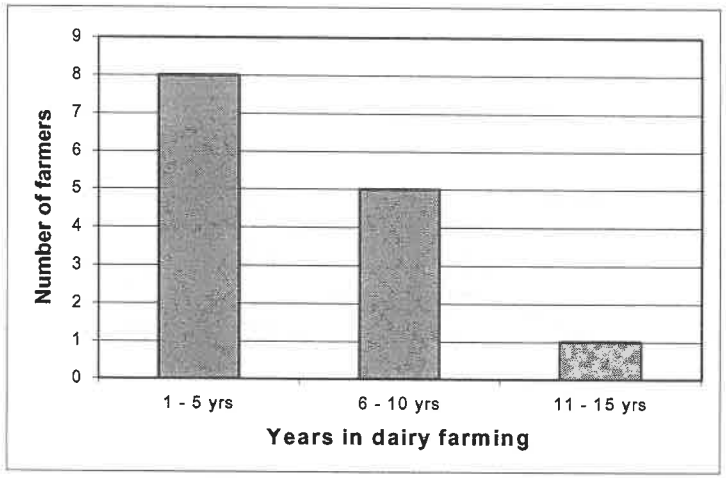

Fig 3. Years in farming

The dairy farming systems used by the 15 farmers varied. In regard to milking duration, eight farmers were milking their cows for fixed periods of 217 or 248 days, five milked for varying periods between 124 and 300 days and one farmer did not know how long the cows were in milk. Nine of the 15 respondents were allowing calves to suckle while they were milking the cows. Six farmers said they were weaning calves naturally, five were weaning calves at the age of not more than 10 days, and the remainder weaned calves at between 62 and 248 days. None of the respondents sold calves immediately after birth, one fed calves with colostrum for three days and then sold them, five raised the heifers and sold bull calves and nine raised both heifers and bull calves.

Identification of individual animals was observed to be inconsistent - respondents used branding, ear notching, ear tags and names to identify their dairy cattle. Record keeping is one of the most important tools for good management. Only one farmer (number 15) was keeping full records In order to monitor a dairy production and select cows, farmers would at least have to keep records of milk production and milk sales. Only five respondents were doing this - the others said they had no idea how to measure the milk as they did not know the capacity of the buckets they used. Only farmer 15 was using machine milking, all the other farmers were milking by hand. Nine of the farmers said they used teat dips and one was using milking salve.

Respondents were asked how they acquired the dairy cattle and 11 respondents mentioned that they bought cattle (mainly from stock auctions) and also bred cattle from their own cows. The breed and herd data are shown in Table 2.

Table 2. Breeds of dairy cattle used by farmers

	Breed of dairy cows	Total number of cows in milk	Total number of bulls used for dairy cows	Total number of dry cows	Total pregnant heifers
1	Friesland	27	1	17	10
2	Cross-bred (Brahman x Friesland)	7	0	9	1
3	Cross-bred (Brahman x Friesland)	7	1	Not sure	Not sure
4	Friesland and Brown Swiss	4	1	2	1
5	Friesland	14	1	3	3
6	Friesland	12	2	5	5
7	Cross-bred (Friesland x Simmentaler x Jersey)	20	1	8	Not sure
8	Friesland	5	1	5	1
9	Friesland, Brown Swiss, and Cross-bred (Brown Swiss x Friesland)	9	1	7	4
10	Friesland and Jersey	0	0	0	0
11	Jersey	2	2	1	0
12	Cross-bred (Bonsmara x Friesland)	13	1	Not sure	3
13	Friesland	6	1	2	0
14	Jersey	3	0	7	0
15	Friesland	90	1	40	48

At the beginning of the investigation, the total number of cattle on the farms ranged from 5 to 188 with an average of 48 cattle and the total number of cows in milk ranged from 0 to 90 with an average of 15 cows. During the interviews about herd composition it was observed that some respondents were not really sure about the numbers of their cattle, and, as some of the interviews were conducted in the afternoon when the cattle were out grazing, it was difficult to count them. This was highlighted when we assisted the farmers in identifying the cows by putting on ear tags. Farmer 15 kept computerised records and his numbers were correct, however they sold a large number of the cows in 2003 and again in 2004. This finding highlights the requirement for development of farm records.

None of the farmers had an adequate level of knowledge of animal diseases, stock remedies and vaccines. Two farmers used herbal medications. Fortunately, eight farmers mentioned that they contacted state veterinarians and animal health officers for assistance with animal health problems.

None of them, however, made use of the private veterinarians who usually service commercial dairy enterprises in South Africa.

Water is a very important resource for production and hygiene in the dairy industry, therefore the farmers were asked about their water sources and water storage. It was found that one respondent had a bore-hole with a solar pump, one respondent got water from a nearby farm, six respondents had bore-holes with electric pumps, four respondents had bore-holes with windmills, one respondent had a bore-hole with a diesel pump and one respondent had a bore-hole with a manual pump (Table 4). Five respondents were only using tanks to store water, five had tanks and a dam or dams and four had dams only. Respondents were also asked if they had electricity in their farms, it was found that 50% (n=7) of the respondents had electricity and the other 50% (n=7) did not have electricity in their farms (Table 3).

Table 3. Water source and infrastructure in dairy farms

Farm number	Main water source	Water storage	Infrastructure	Electricity (Y/N)
1	Bore hole (solar pump)	Tanks	A, B, D, E, G*	No
2	Nearby farm (Buy)	Tanks	A, B, C, D, E,	No
3	Bore hole (electric pump)	Tanks	A, B, C, E, K	Yes
4	Bore hole (electric pump)	Tanks and dam	A, B, D, E, F, I*	Yes
5	Bore hole (windmill and electric)	Tanks and dam	A, B, D, E, F*, H*, I*, K	Yes
6	Bore hole (windmill)	Dam	A, B, D, E, K	Yes
7	Bore hole (diesel)	Dam	A, B, D, E, K	No
8	Bore hole (windmill)	Dam	A, B, D, E, K	No
9	Bore hole (windmill)	Tanks and dam	A, B, D, E	No
10	Bore hole (electric pump)	Dam	A, B, D, E, F, H, I, K	Yes
11	Bore hole (manual)	Tanks	B, D, E, F, K	No
12	Bore hole (electric pump)	Tanks	A, B, D, E, F, I, K	Yes
13	Bore hole (electric pump)	Tanks and dam	A, D, E, F	Yes
14	Bore hole (windmill)	Tanks and dam	A, B, E, I	No

* Not functional, A = Feeding trough, B = Drinking trough, C = Spray race, D = Fenced paddocks, E = Crush pen, F = Milking parlour, H = Milking machine, I = Bulk milk tank, K = Washing/toilet facilities for workers

Water quality was not found to be adequate on many of the farms. This is a difficult problem to address as it appears that many of the boreholes are delivering contaminated underground water. However in some cases the dams they were using were contaminated (Table 4).

Table 4. Water quality tests per farm in 2003

Farmer code	Total plate count per ml	Coliform count per 100 ml	*E.Coli* count per 100 ml
1	4384 [a]	>200 [a]	14 [a]
2	56448 [a]	>200 [a]	<1 [e]
3	5920 [a]	>200 [a]	36 [a]
5	84 [c]	<1 [c]	<1 [d]
6	912 [a]	19 [a]	2 [a]
7	182 [a]	<1 [c]	<1 [d]
8	154 [a]	70 [a]	22 [a]
9	322 [a]	3 [a]	<1 [c]
10	45 [b]	<1 [c]	<1 [d]
11	4 [b]	<1 [c]	<1 [c]
12	338 [a]	3 [a]	<1 [c]
14	322 [a]	3 [a]	<1 [c]
13	27 [b]	5 [a]	<1 [d]
15	0	<1 [c]	<1 [d]

a=out of specification, b=within the range, c=acceptable, d=ideal, e=could not be detected at all. Serious problem

It was found that four farmers threw the cattle manure away, four swept it down a drain, two used it as crop fertiliser, one had a manure dam and four did not dispose of cattle manure. Five farmers were using Rattex (rat poison), two used cats to control rats and eight did not control rats. Four farmers were using a dip spray (an acaricide used for tick control) to control flies, three were using fly traps, five were using fly spray and three were not controlling flies. The information obtained from these questions indicated a deficiency in milk hygiene, which was confirmed at a later stage when milk samples were taken and found to have unacceptably high bacterial counts (Table 5).

Table 5. Results of tests for *Brucella*, *E.coli* and coliforms from bulk tank milk

Farmer code	Coliform count per ml				*E.coli* count per ml				*Brucella* Milk Ring test			
Dates ***	Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4
1	4	TNTC**	125	17	0	0	0	5	-	-	-	-
2	15*	115	7	TNTC**	2	0	0	0	-	-	-	-
3	TNTC**				0				-			
5	24	275	TNTC**	TNTC**	0	0		0	-	+		+
6	0	TNTC**	48	TNTC**	0	0	0	2	-	-	-	-
7	0	TNTC**	TNTC**	TNTC**	0	0	0		+	-	+	
9		54	TNTC**	TNTC**		1	0	0		-	-	-
8	3*	5	5	TNTC**	1	1	1		-	-	-	
12	104	TNTC**	33	TNTC**	0	1	4	0	-	-	-	-
13	TNTC**	TNTC**	TNTC**	58	0	0	0	0	-	-	-	
14		54	TNTC**	TNTC**		1	0	0		-	-	-
15	10	TNTC**	77	152	0	0	1	2	-	-	-	-

*acc = acceptable, **TNTC = Too Numerous To Count, (**Bold**) Unacceptable Serious problem

Milk from bulk tanks was examined on 3 occasions between mid 2003 and mid 2004 The results showed that milk hygiene was unsatisfactory and this was addressed immediately A farmer's day was held with the farmers and a training workshop was held with the relevant extension staff to improve milk quality and lower the bacterial counts. The Brucella positive herds were retested and found that, in the one case, a cow had been vaccinated late, while in the other, a new cow had been bought in with Brucellosis. The state veterinary services placed the latter herd under quarantine and are monitoring the situation after the positive cow was culled.

Cooling of milk and milk storage has an effect on milk quality and milk hygiene. Ten farmers stated that they cooled their milk after milking; the other five did not. Nine farmers were storing their milk in closed buckets, two in a bulk cooler tank, three in milk cans, and one in drums. More than half (n=8) were using refrigerators to cool their milk and two farmers were using a bulk cooler tank to cool milk. Sour milk was sometimes sold for a higher price (sour milk is culturally acceptable in South Africa) or fed to pigs if it was not fit for human consumption. Sour milk has a high bacterial count and this was seen on the bulk milk tank samples.

As a baseline, all cows involved in the project had udder health examinations in 2004. Cows where mastitis was suspected had quarter sampling done and the milk was cultured. Table 6 shows the results.

Table 6. Udder health results per farmer

Farmer number	*S. aureus*	*Staph.* spp	*Streptococcus*	Enterobacteria	*Rhodococcus*
1	+	+		+	
2	+	+			
3	+	+			
5	+	+		+	+
7	+				+
8		+			
9	+	+			
12	+	+			
15	+	+	+		

It appears that *Staphylococcus aureus*, which is a significant pathogen for humans and causes severe food poisoning, is a serious problem on these farms. The mastitic cows identified were treated or culled and are still being followed up. Only two farms still show the presence of S.aureus mastitis.

DISCUSSION

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. Similar findings are described for beef cattle owners by Mokantla et al., (2003).

The interviews revealed that on most farms the system of milking and calf rearing was closer to the traditional management of communal cattle (Bembridge and Tapson,1993; De Leeu and Thorpe,1996; Honhold et al., 1992; Perry et al.,1987; Perry et al., 1984) than specific management for dairy production. Successful intensive and zero-grazing small-scale dairy farms have been described in the literature, however, there are economies of scale in high-input/ high-output systems Bayemi et al.,2002; Bebe,2003; Bebe,Udo and Thorpe, 2002; Kinsey 1993; Presswood et al., 1985; Prinsloo and Keller,2000; Reynolds et al, 1996). Low-input, low-output model has been suggested for smallholder and resource-poor farmers by various authors (De Leeuw and Thorpe,1996; Devendra, 2001; ILCA, 1990; Mack, 1990; Smith, 1995).

South Africa is characterised by a wide variety of infectious diseases of livestock and these can pose a threat if adequate control measures are not in place (Coetzer et al., 2004; De Leeu et al.,1995) .While most respondents were aware of the major diseases of dairy cattle, they knew very little about the prevention and treatment of disease and few practised effective control of internal and external parasites. The high level of Staphylococcus aureus mastitis found initially, raised concerns of possible zoonotic transfer to milkers and consumers. It is probable that cows bought from stock auctions by the respondents were cull cows from commercial herds and entered the system already infected with S.aureus mastitis as the respondents did not know that they should inspect the udders and milk of cows prior to purchase.

CONCLUSIONS

It is essential that more attention be paid to milk safety, as a significant proportion of the target market comprises immuno-compromised people. This study was undertaken to improve the productivity and milk safety of small scale dairy farms. In general the following constraints to effective milk hygiene were shown to be:

- Distances between farms make it difficult for them to have communal collection points so that they share a cooling or bulk milk tank. So every farmer needs their own tank
- Lack of agricultural and veterinary extension on dairy – mainly because the milk safety legislation is governed by the Dept of Health, so health inspectors, many of whom do not have a background in agriculture, are advising the farmers
- A history of sophisticated commercial farming – the veterinarians involved in dairy are consequently in private practice so cannot afford to assist non-paying clients. Small scale farmers do not yet earn enough from their cows to pay veterinary fees.
- Lack of knowledge about medications and their use might result in undesirable residues in milk, which could affect human health. Due to financial constraints, there was fortunately little use of stock remedies.

These have been addressed by enrolling the farmers in the North West Province Mastitis and Udder Health programme and by facilitating mentorship by commercial dairy farmers. It is visualised that the commitment of the state veterinary services to improving udder health and milk quality will have a positive impact on both human and dairy cow health .

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AN INVESTIGATION OF BOVINE ABORTIONS IN THE NORTHERN FREE STATE

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SUMMARY

Eighty one cases of bovine abortion from the Northern Free State area were investigated between 2003 and 2005. The geographical distribution of the samples was examined by a closest allocation to the abortion diagnosis. The viral and mycotic causes seem to prevail in the eastern river basin. Temporal examination showed that the highest incidence was in August. The investigation was based on a post mortem examination, smear examinations and bacteriological cultivation. Virology was done by the OVI and a project was initiated to improve Chlamydial diagnosis. Serology was used to gather further information in some cases. A diagnosis was made in 70 cases (86%). *Brucella abortus* caused 37 (45.6%) of the abortions. All but two of the isolates were *B. abortus* biotype 1. The RB 51 strain was isolated once and Strain 19 once. *Chlamydia* was diagnosed in 9 cases (11%). A diagnosis of *Coxiella burnetti* was made in 4 cases (5%). Bovine viral diarrhoea virus was demonstrated in 3 cases (3%). One abortion was attributed to *Arcanobacterium* and one to Trichomoniasis. Suspected viral causes of abortion were diagnosed on 5 post mortem examinations. Mycotic abortion was suspected in 4 cases. *Anaplasma marginale* were visible in one case in red blood cells of the foetus. In 5 cases it was found that the foetus was probably alive in the birth canal and the death was due to dystocia.

Diagnostic techniques, peculiar findings, possible improvements and confounding considerations are discussed.

INTRODUCTION

The examination of bovine reproductive failure and specifically abortions is an important function of the Kroonstad Veterinary Laboratory. The samples were submitted from the Northern Free State area in districts where both dairy and beef cattle farming are well established.

MATERIALS AND METHODS

Aborted foetuses were submitted by farmers, private and state veterinarians or animal health technicians of the Free State Division of Veterinary Services. When they were available placenta samples were also examined.

The history was recorded at registration and a post mortem examination was conducted. A set of two impression smears were made of the abomasum mucosa, the lungs and the kidneys. Organ samples were collected for bacteriology. A piece of abomasum was tied off, a liver, lung and kidney sample were cut out and subjected to culture on selective media. When submitted placenta samples were also cultured and smears were also examined.

A spleen and lung sample collected in a jar with phosphate buffered saline was taken for polymerase chain reaction tests for bovine viral diarrhoea virus. The examinations were done at the Onderstepoort Veterinary Institute.

Bacterial samples were cultured for *Brucella* on Farrels medium in 10% CO_2 (Oxoid Carbon dioxide system Br9) for up to 14 days. *Brucella* typing was done on CO_2 requirement as well as basic fuchsin-, erythritol- and penicillin sensitivity.

Trichomonas foetus broth was inoculated and examined by dark field microscopy after cultivation at 27°C for 7 days.

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BTA was inoculated and cultured in jars with Campylobacter Gas (Microaerophilic Oxoid Br56).

BTA was inoculated and cultured in 5% CO_2.

One impression smear of each organ was stained with the Stamp's method and one with the Giminez method. The smears were examined with oil immersion under a light microscope.

Eighteen samples were submitted in *Chlamydia* transport medium for a Chlamydial diagnostic project at the OVI.

Brucella abortus was diagnosed on the finding of typical intracellular partly acid fast organisms after staining of foetal organ impression smears with the Stamp's method or the identification of the organism on cultures from foetal samples. A diagnosis of Chlamydiosis was made after groups of intracellular elementary bodies were identified on Giminez stained smears of foetal organs. In two cases seroconversion of the cow was also taken into account.

Coxiella burnetti was incriminated when partly acid fast organisms were seen extracellularly and widely spread over impression smears after Stamps staining.

Mycotic abortion was reported as suspected when typical skin lesions were seen, yeasts were present in conspicuous numbers on impression smears and no other infection was demonstrated.

A diagnosis of suspected viral abortion was made on finding congenital abnormalities in the nervous system in the absence of any evidence of other infections.

Localized swelling of the tongue and head, ribcage bruises, tracheal petechiae and in some cases aerated lungs, lead to the diagnosis of dystocia.

The geographical distribution of the samples was examined by a closest allocation to the abortion diagnosis.

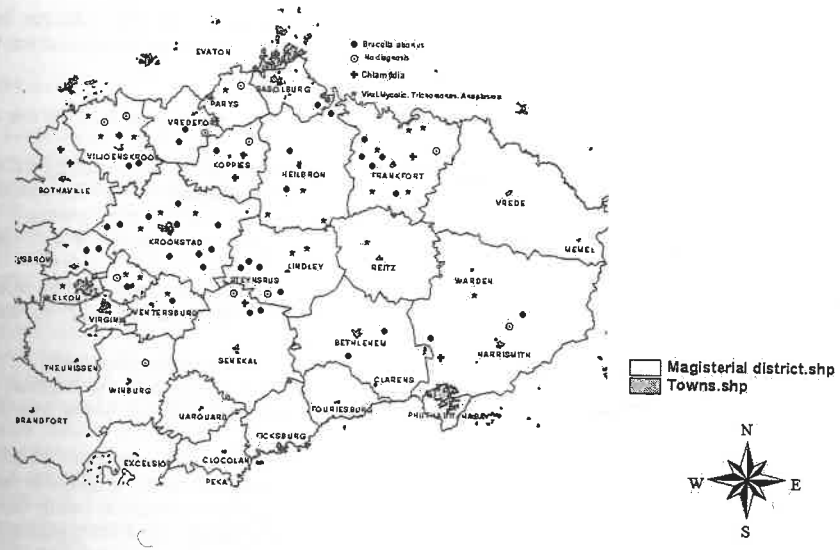
RESULTS

The diagnoses of the examinations over the 26 month period April 2003 to June 2005 are reflected in Table 1.

Table 1. Diagnosed causes of bovine abortion, April 2003 to June 2005

Cause of abortion	Number
No diagnosis made	11
Brucella abortus Biotype 1	35
Brucella abortus Vaccine strains	2
Chlamydia	9
Suspected Viral abortions	5
Dystochia	5
Coxiella	4
Mycosis	4
Bovine Viral diarhoea	3
Trichomonas foetus	1
Arcanobacterium pyogenes	1
Anaplasma marginale	1
TOTAL	**81**

Figure 1. Origin of the abortion samples from the northern Free State examined at the Kroonstad Veterinary Laboratory 2003 to 2005.



When an allocation to the closest abortion diagnosis was examined, it appears as if the viral and mycotic causes seem to prevail in the eastern river basin. An inverse distance weighting interpolation was also done, although it would not strictly be applicable because the data was not continuous. The same picture resulted only slightly more rounded.

The temporal distribution shows peaks in August followed by October and April, with the lowest submissions in February followed by January and November.

DISCUSSION

The origin of the samples could have been influenced by the performance of the Animal Health Technician active in the area or the effect of extension done as well as the level of farmers union activity and management level of farmers in the area.

Twenty five percent is a common figure quoted for the success rate of abortion investigations. The fact that *Brucella* was responsible for 45% of the abortions and that isolations and smear examinations enabled us to confirm those cases contributed to a higher success rate. By making a diagnosis based on tests with limited specificity or nominating suspected causes the accuracy of the investigation was compromised but owners were enabled to act on possible threats.

The later stage of abortions commonly found in cases of Brucellosis might have contributed to the high percentage of *Brucella abortus* diagnosed, as the foetuses would have been easier to find.

Serological examinations of herds after a diagnosis of *Brucella abortus* had been made on a foetus sample gave valuable confirmation in many cases. Culture success on lochia and milk also strengthened the specificity of *Brucella* procedures.

Inadequate vaccination was recorded in slightly more than half of the cases of *Brucella* abortions. In the context of zoonotic diseases one would have to assume that dangerous levels of exposure of humans in the area exist.

Chlamydia appears to be important and this finding was supported by a private veterinarian who had obtained confirmation in his investigations on histopathology of lung tissue after Giminez staining.

Virological diagnosis remains a problem as abortions take place long after infection has subsided. Demonstration of exposure to Bunyaviridae is strongly considered as an improvement to abortion examinations.

Dystocias were commonly mistaken for abortions.

The relatively high incidence of *Coxiella* is alarming. Very little extension work on the risk of the disease is done in the area.

Mycosis diagnosis is widely open to criticism. The fact that many healthy calves are apparently borne with fungal infections complicates the finding. In the absence of other infections the presence of typical skin lesions or large numbers of yeasts in organ smears could not have been ignored.

Bovine viral diarrhea virus was expected in the light of serological evidence of very high exposure in the area.

The diagnosis of one case of *Trichomonas foetus* prove that it is present as indicated by sheath washings, while the absence of *Campylobacter fetus* raises the question why so little *Campylobacter* is seen in so many sheath washings.

Arcanobacterium pyogenes was striking in smears of lochia and foetal organs in one case.

High parasitaemia of *Anaplasma marginale* in foetal red blood cells lead to the diagnosis of *Anaplasma* as the cause of one abortion. The endemic nature of the disease in the area will probably sustain it as a low level cause of abortion.

Specific investigations into the occurrence of *Neospora* will be attempted as an improvement of abortion diagnosis. Serology follow up examinations could contribute a lot to establish herd situations. Currently histopathology and virology are weak areas in the investigations mainly due to financial restrictions, but also due to delays in results. Faster virus demonstration techniques rather than virus isolation could be a solution.

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AVIAN INFLUENZA IN OSTRICHES: FINDINGS OF AN EPIDEMIOLOGICAL INVESTIGATION IN THE WESTERN CAPE PROVINCE

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SUMMARY

South Africa did not escape the scientific debate and consequences following the detection of avian influenza in Asia in 2003 and the spread thereafter to other parts of the globe. The disease was subsequently also detected in South Africa in ostriches on a farm in the Eastern Cape Province during 2004. A national survey in poultry and ostriches was immediately sanctioned to detect the possible presence of the disease in other provinces of the country following the laboratory confirmation of the disease in the Eastern Cape Province.

The survey conducted in the Western Cape detected antibodies to H5 avian influenza virus on approximately 50 farms in the province. However, the sampling strategy initially embarked upon to accommodate practical constraints did not in all instances truly reflect the prevalence of the sero-reactor entities. Samples were, for example, mostly collected from a specific group of birds on a farm, particularly slaughter birds, as these were more easily accessible and manageable. The time of the survey also coincided with the breeding season and farmers were very reluctant to allow their breeder birds to be sampled. The non-random sampling strategy also impacted negatively on the interpretation of the results. To rectify the problem a revised sampling frame was designed to ensure randomness taking into account the practical situation and management practices on a farm. A follow-up survey was therefore conducted during March and April 2005 to obtain an absolute representative sample of ostriches in the province.

The results of the surveys offered some interesting inferences, the most important being the lack of knowledge of the disease characteristics and epidemiology of avian influenza in ostriches, making the interpretation of results and the subsequent decision making process for dealing with the disease, extremely difficult.

Towards the end of the 2005 survey, several farms throughout the province were subjected to an intensive epidemiological investigation to identify relevant risk factors and risk mitigation procedures that could be applied to minimise the spread of the disease. Farms visited included sero-positive as well as neighbouring sero-negative farms with the main objective to determine possible epidemiological variants such as differences in management, geographical characteristics, and the role of migratory wild bird species in the dissemination of the disease.

INTRODUCTION

Highly pathogenic avian influenza (HPAI) has never occurred in South Africa in the domestic poultry industry. However, HPAI virus of the H5N2 subtype was isolated from ostriches (*Struthio camelus*) on a farm in the Eastern Cape Province in July 2004.

The first isolations of influenza viruses from ostriches were viruses of the H7N1 subtype, but of low pathogenicity in chickens, which was obtained during an outbreak in the Western Cape Province of South Africa during 1991. During this outbreak young ostriches between 5 days and 14 months of age were affected worst, while very few adult ostriches developed clinical signs. Chicks under one month of age died peracutely with mortality rates often exceeding 80 per cent. Young ostriches between the ages of 2 and 8 months had mortality rates between 15 and 60 per cent (Allwright, Burger, Geyer & Terblanche 1993). Since then H6N8 (1998) and H10N1 (2001) avian influenza virus subtypes were diagnosed in ostriches in the Western Cape. Both of these were

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classified as low pathogenic avian influenza (LPAI) subtypes (Olivier 2005). During 1998 a H6N8 virus was isolated from an Egyptian Goose (*Alopochen aegyptiacus*) from the same area as where this specific virus subtype was isolated from an ostrich. Similarly, a virus of the H5N2 subtype was isolated from an Egyptian Goose in the Western Cape Province during 2004 two weeks prior to the outbreak in ostriches in the Eastern Cape. Unfortunately the virus isolated from the Egyptian Goose was lost and no further classification studies could be conducted (Olivier 2005). These findings emphasize the critical need for further investigation into the role of wild bird populations in the epidemiology of avian influenza.

In 2004, during the national avian influenza survey, 50 farms in the Western Cape Province tested serologically positive for H5 notifiable avian influenza (NAI) using the HI test. On none of the farms surveyed were any clinical disease or symptoms or mortalities related to avian influenza detected. Also, in spite of intensive sampling, no virus could be detected with either PCR or virus isolation. It was decided to repeat the survey during 2005 in order to obtain a better representative sample. Again no clinical disease, related mortalities or virus could be found, but the improved sampling strategy allowed for interesting epidemiological deductions. Following from previous studies indicating that avian influenza viruses are present in some of the wild aquatic bird populations in the Oudtshoorn area, several farms were visited to determine the degree of exposure of ostriches to wild birds (Pfitzer, Verwoerd, Gerdes, Labuschagne, Erasmus, Manvell & Grund 2000, Olivier 2005).

MATERIALS AND METHODS

Study population

Samples were taken from a representative number of ostriches on all the ostrich farms in the Western Cape Province during two time periods, August 2004 to February 2005 and March to May 2005. This constituted 17 675 samples on 463 farms during the first survey and 38 581 samples on 761 farms during the second survey.

In addition 14 farms were visited towards the end of the survey to determine possible differences in management practices that can contribute to an increased or decreased risk of infection. These farms were selected according to their test results and location (11 farms had a high number of sero-reactors, and 3 farms tested sero-negative which were all surrounded by sero-positive farms).

Study design

The basic epidemiological formula for the detection of disease was used for the calculation of sample sizes during both surveys: $n = \left[1-(1-\alpha)^{\frac{1}{D}}\right]\left[N-\frac{D-1}{2}\right]$ (Dohoo, Martin & Stryhn 2003).

At the onset of the 2004 survey, the assumption was made that the disease will spread rapidly amongst ostriches, as is the case with poultry. It was assumed that if the disease were present in an ostrich population, the population would have a minimum expected prevalence of 20 per cent. This was compatible to the OIE guidelines for the surveillance of NAI in an establishment (OIE 2004). Using the above formula with 95 per cent confidence intervals, a sample size of 14 ostriches was selected, which was increased to 16 ostriches since this was more convenient for the diagnostic procedures in the laboratory. As the survey progressed it was evident that the disease does not spread rapidly among ostriches and the minimum expected prevalence was adjusted to 10 per cent, and 30 samples were then collected per farm. On farms where sero-reactors were found, the sampling size was increased to 60, which corresponds to a minimum expected prevalence of 5 per cent. Individually identified birds were tested repeatedly. During the 2004 survey the slaughter birds (5-14 months) were targeted as these were assumed to be more likely subject to exposure to risk factors. However this assumption limited the epidemiological deductions that could be made from the results and an epidemiology unit had to be redefined for the 2005 survey.

Following consultation with local and international experts, the second (2005) survey was therefore re-designed to detect virus and/or antigen within an epidemiological unit at a prevalence

of at least 5 per cent and antibody for H5 NAI at a prevalence of 10 per cent. An epidemiological unit was defined as a group of ostriches that is managed separately from other groups on the farm and thus with a different risk profile than the other groups. According to this definition three possible epidemiology units could be identified on a farm: 0-4 month old chicks (normally held in intensive rearing systems), 5-14 month old slaughter birds (mostly intensive feedlot situations) and breeders (extensive range situations). A stratified random sampling approach was adopted with the above-mentioned three groups constituting the strata and random samples were taken from all the inhabited camps on a property, weighted according to group size in a specific camp. To ensure the correct sampling size for each farm, the individual farms were pre-visited by officials to obtain a camp-by-camp census captured on a specifically designed census form. The data was captured at a central epidemiology centre into a spreadsheet, which was calibrated to calculate the sample sizes required from each camp on the farm. A printout of the sampling frame was thereafter provided to the sampling teams. This translated to approximately 60 samples (cloacal swabs) per epidemiology group (180 per farm) for virus detection and approximately 30 serum samples per epidemiology group (90 per farm) for serology.

Sampling procedures

Blood was collected from the jugular vein in 7ml SST yellow stopper gel tubes, containing clot activator. These were centrifuged before transport by courier to the relevant diagnostic laboratory, which was either the Provincial Veterinary Laboratory in Stellenbosch, ARC-OVI or Allerton depending on available capacity of the laboratories on a specific day.

Cloacal swabs were collected and 5 swabs pooled in Phosphate Buffered Saline (PBS) in cryotubes before their dispatch on ice within 12 hours of collection by courier to the Onderstepoort Veterinary Institute PCR Laboratory.

Analytical procedures

The serum was tested for the presence of H5 antibody by the beta haemagglutination inhibition (HI) test according to the procedure described in the 5th edition of the World Animal Health Organisation (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2004). The ostrich sera were adsorbed with packed chicken red blood cells to remove any interfering factors. The antigen used was derived from the H5N2 virus isolated from infected ostriches in the Eastern Cape Province in 2004.

The swabs were tested for the presence of antigen by reverse transcriptase polymerase chain reaction (RT-PCR) assay according to the Starick method, which uses primers to the matrix gene (Starick, Rømer-Oberdørfer, & Werner 2000).

There is however, no published or recorded information available on the sensitivity and specificity of these two diagnostic methods in ostriches.

Data analysis

The data was captured into a Microsoft Access© database to record all relevant epidemiological information and a Microsoft Excel© spreadsheet for statistical analysis.

RESULTS

Table 1. Comparison of results from the first survey (August 2004 to February 2005) and second survey (March to May 2005)

	First survey	Second survey
Number of farms tested	463	761
Number of farms positive: antibody	50	124
Number of farms positive: antigen	0	0
On-farm sero-prevalence: average	16.08%	7.82%
On-farm sero-prevalence: median	12.50%	6.05%
On-farm sero-prevalence: range	0% - 60%	2 – 42.55%
Number of serum samples tested	17 675	38 581
Percentage serum samples positive of all samples tested	7.39%	1.68%
Number of swab pools tested	228	3 189
Number of swab pools positive for antigen	0	0

Table 2 depicts the additional epidemiological information that could be obtained during the second survey.

Table 2. Sero-prevalence in the different epidemiological units during the survey (March to May 2005)

	Chicks (0-4 months)	Slaughter birds (5-14 months)	Breeders (>14 months)
Number of samples tested	15 126	14 664	8 791
On-farm sero-prevalence: average	0%	9.57%	11.43%
95% Confidence interval	0%	9.27% - 9.93%	10.83% - 11.97%
On-farm sero-prevalence: median	0%	4.00%	8.70%
On-farm sero-prevalence: range	0%	0 – 42.86%	0 – 85.71%

All of the 14 farms visited for epidemiological analysis, utilised concrete watering troughs (similar to those used in cattle and sheep farming) and vehicle tyres for holding feed. The 14 farms represented 11 sero-positive and 3 sero-negative farms. One out of the three sero-negative farms experienced no problem with wild birds on the farm, but on the other two farms there was a high degree of contact between the ostriches and wild birds. The bio-security measures on the latter two farms differed significantly from those implemented on the 11 sero-positive farms. The most noticeable was the regular (at least once a week) cleaning of water troughs and tyre feeders.

DISCUSSION

The explanation for the higher number of farms tested during the second survey are twofold. Firstly, due to farming practises characteristic of the ostrich industry, some farms did not have any ostriches on them during the first survey and were thus omitted from testing. Secondly, and more importantly, some farmers utilise different land areas under the same name and registration number, even though these farms are sometimes far apart. During the first survey these farms were tested as

a single unit, but during the second survey it was decided that the farms should be tested separately in order to gain more information on the exact geographical location of the disease.

The higher number of antibody positive farms (10.80 per cent of farms tested in the 2004 survey versus 16.29 per cent of farms tested in the 2005 survey) could raise some concerns regarding the evolvement of the disease in the area during the period August 2004 to May 2005. However, there are several facts proving the contrary. The average on farm sero-prevalence decreased from 16.08 per cent to 7.82 per cent and the total percentage of positive samples decreased from 7.39 per cent to 1.68 per cent. This together with the fact that no antigen could be detected and no chicks 4 months or younger tested antibody positive, suggests that the increased number of farms with antibodies should rather be attributed to the increased number of farms tested and the improved sampling strategy employed.

The low percentage of positive samples in total during both surveys (7.39 per cent and 1.68 per cent respectively), as well as the low average on farm sero-prevalence, suggests that the disease did not spread significantly between farms or even between ostriches on a farm. Comparing this data with the findings during the 1991 outbreak of H7N1 in ostriches, where mortalities in young birds reached 80 per cent, it appears that the virus subtype responsible for the H5 antibodies in the Western Cape Province could be classified as a virus of low pathogenicity for ostriches.

The average sero-prevalence amongst breeder birds (11.43 per cent) is significantly higher ($p<0.05$) than the average for slaughter birds (9.57 per cent). This could indicate an increased exposure to risk factors among the breeder birds but also a history of previous exposure as it is uncertain how long detectable antibody titres remain after viral challenge. These birds are normally kept under extremely extensive conditions on an ostrich farm and during the breeding season (May to January) they are paired into separate field camps. Human exposure and interventions are restricted to a minimum resulting also in the undisturbed access of wild birds to feed and water in these camps. Chicks of less than 4 to 5 months of age are normally reared in a less extensive environment and are frequently visited by humans, creating an unsuitable and disturbing environment for wild birds. This might explain why none of the 15 126 chicks tested had detectable antibodies. Although, the high mortality amongst chicks during the 1991 H7N1 outbreak suggests that if a NAI virus were highly pathogenic to ostriches, even this young group would be affected.

The majority of reactor farms were concentrated around rivers and riverine areas. Wild migratory birds and other waterfowl are in abundance in these areas and accumulate in vast numbers on the ostrich farms. Previous studies confirm that these birds are the most probable source of viral challenge (Pfitzer, Verwoerd, Gerdes, Labuschagne, Erasmus, Manvell & Grund 2000, Olivier 2005). They tend to graze with ostriches on irrigated pastures and concentrate in great numbers around the watering troughs and feeders where they contaminate the ostrich water and feed with faecal material. The exposure of ostriches to viral challenge could be minimised by applying bio-security measures aimed at reduced contact between wild birds and ostriches. The most important of these is the regular cleaning of watering and feeding troughs with a virocidal agent. It seems that such a management practise reduces the viral load in the environment and subsequently prevents the infection of ostriches. Other management practises such as the utilisation of "wild bird unfriendly" water and feeding troughs could also be employed. The concrete troughs and vehicle tyres that are mostly used on ostrich farms create an ideal environment for social gathering of migratory birds. Smaller water troughs that are elevated off the ground and having sharp edges as well as self-feeders will discourage wild birds from making use of these resources. Terrestrial birds, e.g. hadeda (*Bostrychia hagedash*), African sacred ibis (*Threskiornis aethiopicus*), various dove species, etc., also have extensive contact with the ostriches and the possible role of these birds in the spread of the disease needs to be investigated.

CONCLUSION

Serological findings and intensive epidemiological investigations indicate that there was no active H5 NAI virus circulating since at least 4 months preceding the second survey.

The slow spread of the H5 AI virus, judging from the sero-prevalence also suggests that the virus that affected the Karoo region of the Western Cape was not highly pathogenic to ostriches.

It is internationally accepted that wild waterfowl play a major role in the global dissemination of avian influenza and therefore also posing a constant threat of infection of ostriches with AI viruses due to their close contact with these birds (Martin, Sims, Lubroth, Slingenbergh & Pfeiffer 2005, Senne, Suarez, Pedersen & Panigrahy 2005, Brown, Banks, Manvell, Essen, Slomka, Londt & Alexander 2005). Although no vaccination has ever been implemented, it needs to be considered to limit virus excretion due to this continuous viral challenge.

Bio-security measures should be improved on all ostrich farms and should specifically be aimed to minimise the contact between ostriches and wild birds.

The epidemiological investigations during both surveys also indicate that ostriches do not necessarily display a similar response as poultry to avian influenza virus infection. It is therefore questionable and not scientifically justifiable to extrapolate without reservation the findings from research on the disease in poultry to ostriches in an attempt to try and explain the occurrence of disease in ostriches and to formulate control strategies. Further research on the epidemiology and pathogenicity of AI in ostriches is urgently needed including research on the role of wild waterfowl and terrestrial birds.

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THE 2005 OUTBREAK OF MARBURG HAEMORRHAGIC FEVER IN ANGOLA – WHAT NEW LESSONS HAVE BEEN LEARNED?

J. Paweska[1]

SUMMARY

Filoviruses are amongst the most lethal pathogens to infect man. They are classified as "Biosafety Level 4" agents with a high mortality rate, person-to-person transmission, potential aerosol infectivity, and absence of immunoprophylactic and chemotherapeutic measures. Rapid travel by humans and animals within the incubation period of these viruses presents a considerable risk for their introduction into non-endemic areas. To date there have been 7 confirmed outbreaks of Marburg (6 in Africa) with the most recent being in the Uige Province of north-western Angola and confirmed on 21 March 2005. National and international health teams were deployed soon after the outbreak was identified to assist with infection control, social mobilization activities, case management, laboratory diagnosis, surveillance and monitoring of contacts, funerals and burials, and epidemiological investigations. Despite this the Angolan outbreak of Marburg haemorrhagic fever is now the largest and deadliest on record. The outbreak is reported upon as it unfolded.

INTRODUCTION

Despite intensive efforts, the natural history of filoviruses (Order *Mononegavirales*, family *Filoviridae*) remains unknown. Diverse taxa have been suggested as potential reservoirs, including bats, rodents, arthropods, and plants (1, 3, 5, 8, 12, 13). Since the first isolations of Marburg virus in 1967 in Germany (7) and Ebola virus in 1976 in Zaire (4) following epidemics in humans of a moderate to highly fatal haemorrhagic disease in these countries, until very recently, outbreaks caused by filoviruses were rare (9). However, simultaneous Ebola outbreaks in humans, great apes, and other primates, have occurred each year since 2001 in Gabon and Congo Republic (6, 14). It is noteworthy that for the first time outbreaks in humans caused by the two members of the family *Filoviridae* emerged simultaneously in Africa in 2005: Marburg haemorrhagic fever in west-northern Angola and Ebola haemorrhagic fever in the Republic of Congo, in the Cuvette-Ouest region near the Gabon Border. To date there have been 7 confirmed outbreaks of Marburg with the most recent being confirmed in the Uige Province of north-western Angola on 21 March 2005 (Table 1).

BRIEF HISTORY OF THE 2005 MARBURG OUTBREAK AND ITS CONTROL (2, 10,11, 15)

10 March 2005: The WHO Epidemiological Focal Point for childhood immunization in Angola approaches the Special Pathogens Unit at the National Institute for Communicable Disease (SPU NICD), Sandringham, South Africa to test specimens from fatal haemorrhagic cases among children hospitalized in Uige Province in northern-west Angola, and from a nurse who had died after taking care of some of the patients. The SPU-NICD which houses the only maximum-security laboratory (BSL-4) on African continent could not assist with laboratory testing as this facility is being shut down since April last year for major renovation and upgrading. The Unit assisted, however, in the shipping of specimens from Angola to the Special Pathogens Branch of the Centres for Disease Control and Prevention (CDC), Atlanta, USA.

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Table 1. Marburg disease outbreaks (excluding 3 laboratory infections)

Year	Location	Deaths/Cases	% Mortality
1967	Germany Yugoslavia	7 / 32	22
1975	RPA	1 / 3	
1980	Kenya	1 / 2	
1987	Kenya	1 / 1	
1998-2000	DRC	126 / 154	82.0
2005	Angola	351 / 312*	88.9

* WHO Marburg update on 23 July 2005

21 March 2005: The CDC, Atlanta, US, identifies Marburg virus as the cause of deaths with severe haemorrhagic manifestations in an increasing number of patients, and linked mostly to a single pediatric ward in the main hospital in Uige Province, northern Angola. A retrospective epidemiological analysis, embracing the period 13 October 2004 - 23 March 2005, identified 102 cases in the outbreak. Of these, 95 had been fatal; about 75% occurred in children under 5 years of age (Fig. 1). In adults cases included a small number of health care workers. The predominant symptoms included fever, haemorrhaging, vomiting, diarrhea, jaundice and headache (Fig 2). Diagnostic confirmation prompted a large-scale international response that began the day after the CDC's findings were publicised. The World Health Organization (WHO) outbreak response team supported the Ministry of Health in Angola (AMH) in their efforts to control the outbreak, and included technical support for case management, contact tracing and surveillance, infection control and raising awareness of the disease in the community. Further technical support was provided promptly by experts from the Inter-Country Programme for Southern Africa, by the Regional WHO Office for Africa, by many institutions in the Global Outbreak Alert and Response Network (GOARN), by several other organizations including Médecins Sans Frontières (MSF) from Belgium, France, Holland and Spain, by UNICEF, by the World Food Programme, and by other humanitarian aid organizations.

29 March 2005: 124 cases (117 fatalities) have now been reported from the Uige, Cabinda, and Luanda provinces; all had originated from Uige. Infectious disease control experts from the UK and from South Africa have begun to provide on-site assistance in infection control in Luanda and in Uige as well as the training of health care staff in all provinces. Most urgent was the need to disinfect hospital wards (Fig. 3) and homes where patients had died, to collect and bury corpses, to intensify social mobilization activities, and to provide logistic support and equipment. On 30 March, the Canadian National Microbiology Laboratory arrived in Angola, followed soon by CDC, Atlanta team to establish mobile laboratories in Uige, and Luanda (Fig. 4).

2 April 2005: 163 cases (150 fatalities) have now been reported, and are from the provinces of Uige, Luanda, Cabinda, Malange, and Kuanza Norte; all cases are thought to have originated in Uige. The WHO mobile surveillance teams in Uige continue to investigate unconfirmed reports and to search for additional cases with the Canadian mobile laboratory in Uige supporting these activities. More than 100 contacts were followed up. The WHO is working with the AMH to finalize a national plan of action for outbreak control, but its implementation will require significant assistance from the international community.

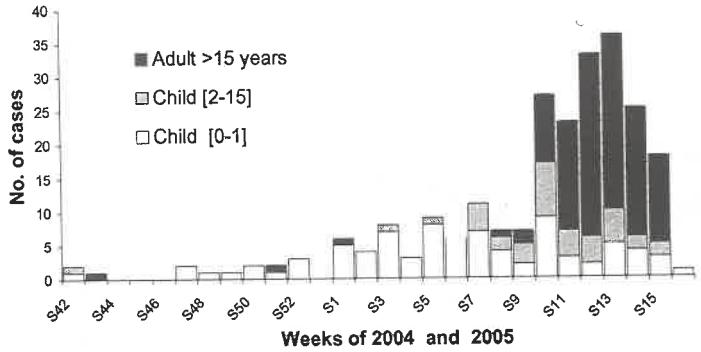

Figure 1. Age distribution among cases of Marburg viral haemorrhagic fever in Uige Province, Angola - October 2004 to April 2005 (n = 227)

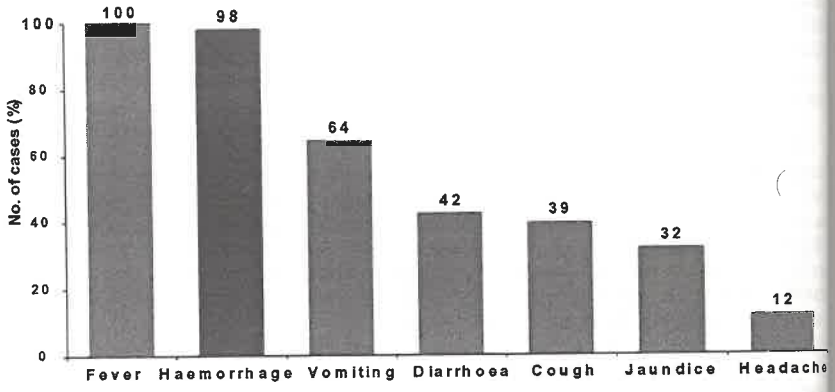

Figure 2. Symptoms and signs of patients with suspected viral haemorrhagic fever in Uige Province, Angola – 13 October 2004 to 24 March 2005 (n=104)

Figure 3. Infection control measures in provincional hospital in Uige, Northern Angola where the 2005 Marburg outbreak was first regonized – health care workers in protective clothing are burning disposable waste in field "drum" incinerator using diesel fuel.

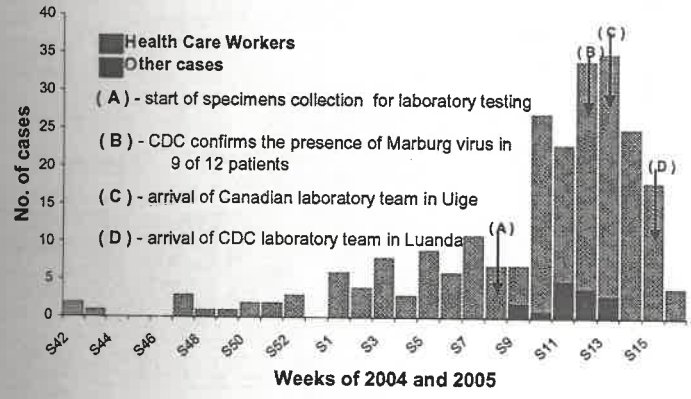

Figure 4. Cases of Marburg viral haemorrhagic fever in Uige Province, Angola - October 2004 to April 2005 (n = 227)

5 April 2005: 181 cases (156 fatalities) have now been reported. While children under the age of 5 years initially accounted for around 75% of cases, recent cases include an increasing number of adults.

6 April 2005: 200 cases (173 fatalities) have now been reported; Kuanza Sul has reported its first case, bringing the number of affected provinces to six, all concentrated in north-western Angola. The WHO has now established an international network of expert laboratories for the diagnosis of Marburg and other viral haemorrhagic fevers VHF). Within this network, laboratories in Canada, Germany, South Africa, and the USA are providing diagnostic support in the Angolan outbreak. The Canadian mobile laboratory is now fully operational in Uige; their results are being used for improved case management and contact tracing.

First joint outbreak assessment: the government of Angola and the WHO released a joint outbreak assessment revealing great concern about further developments; but they remain confident that the outbreak can be brought under control. However, the features of Marburg haemorrhagic fever (MHF) and local conditions in Angola render the implementation of effective control measures extremely difficult. Not only is the Angolan outbreak already the largest on record, and with the highest fatality rate, but is also the first to occur in an urban setting, reaching a very high transmission level. Uige, which remains the epicentre of the outbreak, has 500,000 inhabitants; Luanda, where some cases have occurred, has a population of close to 3 million. Thus, a top priority is to prevent the virus from becoming established in densely populated urban or peri-urban environments. As the incubation period of Marburg disease could be as short as 3 days, rapid and efficient contact tracing is vital towards containing the outbreak. The effective management of contacts needs timely isolatation of cases prior to the onset of symptoms, to limit the risk of further transmission. Other urgent needs include the protection of front-line staff, the strengthening of infection control in isolation wards, the improved transportation of suspected cases to designated isolation wards, and the education of the public to encourage protective behaviours and to improve compliance with control measures.

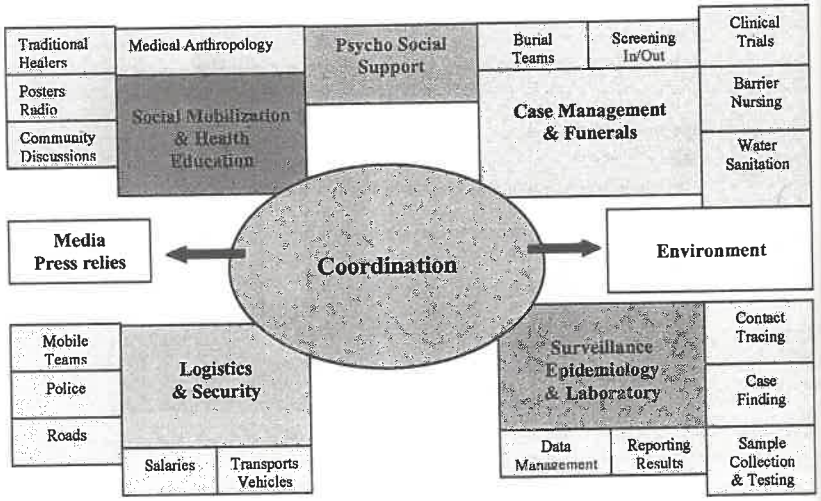

Figure 5. WHO strategy for controlling the 2005 Marburg outbreak in Angola

Decades of civil unrest have left Angola with a severely impaired health infrastructure, a hospital system in dire need of basic equipment and supplies, inadequate communication and transportation systems, and a population weakened by economic hardship. These factors hamper disease containment efforts, which depend on active surveillance for rapid detection of cases, and isolation in specially designated and equipped facilities, and the rapid tracing of contacts. It is also important to stress that deaths amongst doctors and nurses have undermined the morale of hospital staff already working under difficult circumstances.

Based on experience gained in previous filovirus outbreaks, the above relatively straightforward control measures can successfully interrupt the chain of transmission. However, their effectiveness depends upon a sustained application, and supported by efficient surveillance, which in turn, requires suitable communication and transportation systems. Unfortunately, are not available in Angola and cannot be established quickly. Also, landmines remain scattered over a vast area making transportation by rail and road dangerous, often requiring staff and equipment to be airlifted. Intensified surveillance in Uige has revealed that some patients are dying within the community, creating an urgent need to organize services for their safe collection and burial. Cases in front-line health care workers indicate the need for protective equipment and for training in their use. The manifestations of VHF in patients, and the high fatality rate, cause great anxiety in affected populations; this increases the risk of people fleeing from affected to unaffected areas, thereby contributing to the wider spread of Marburg disease. Furthermore, control measures are socially disruptive, and so add to public unease; indeed, in Uige, some persons are reluctant to seek treatment or to remain in hospital. There is an urgent need to strengthen the hospital system and to restore public confidence.

7 April 2005: 205 cases (180 fatalities) now reported including the first 6 from Zaire Province; this brings the number of affected provinces to seven, all concentrated in north-western Angola. Mobile surveillance teams in Uige were forced to suspend operations on this day when vehicles were attacked and damaged by local residents. Despite several fatalities reported on the following day the teams were unable to investigate the cause of death or to collect the bodies for burial. The situation did not improve subsequently, and urgent discussions were held with the provincial authorities to find solutions. The dramatic symptoms of MHF, and frequent fatalities, are creating a high level of fear, aggravated further by a lack of public understanding of the disease. Moreover, because the disease has no cure, hospitalisation is not associated with a favourable outcome, further eroding confidence in the medical care system. The WHO is familiar with such reactions, seen during previous outbreaks of Ebola (a closely related haemorrhagic fever). Two medical anthropologists are now in Uige and will be joined soon by experts in social mobilization from Angola, the Democratic Republic of Congo, and Mozambique. Public compliance with control measures is not expected to improve in the absence of intense campaigns to educate the public about the disease. In African settings, one of the most important factors in controlling VHF is the engagement of affected communities as partners in the control effort. To achieve this, local belief systems about the causes of the disease, and traditional rituals for mourning the dead, must be respected. When the public understands and accepts a few simple messages – avoid contact with blood and other fluids when caring for the ill, and to not touch the bodies of the deceased – only then can transmission within the community be stopped and the outbreak brought under control.

8 April 2005: The WHO launched an international appeal, through the United Nations, for funding to support the emergency response to the outbreak of Marburg in Angola. US$ 2.4 million is needed for the AMH to intensify operations in the field. Specialized international staff and equipment have been deployed rapidly and measures are beginning to have an impact; however, the control of the outbreak will require intensified and sustained technical support, and additional supplies. The need for adequate personal protective equipment is particularly urgent. Increased field coordination of technical, operational and logistic support is likewise needed.

Figure 5. Collection of specimens from a patient who died in Uige hospital during the 2005 outbreak of Marburg in Northern Angola.

Figure 6. Infection control measures during burial of a patient who died during the 2005 outbreak of Marburg in Uige, Northern Angola. 0.5% chlorine is used for spraying the body bag inside a coffin.

9 April 2005: 214 cases (194 fatalities) now reported; Uige Province remains the epicentre of th outbreak, accounting for almost 90% of the cases and deaths. Mobile surveillance teams hav resumed operations following intensive campaigns to improve public understanding of the disease The campaigns have benefited from the support of the provincial governor and officials from th health department, who have personally visited affected communities. Religious leaders are als helping to sensitise the public. Some 360 contacts are being followed up but improvements ar needed to detect cases earlier, to ensure their isolation and supportive care, and to locate and manag contacts. The WHO is concerned that deaths are continuing to occur within the community. The car of patients by family members without adequate protective equipment greatly increases the risk o further transmission. Staff at Uige's provincial hospital also need training and equipment to reduc risk as routine surgical and laboratory procedures might endanger staff and other patients. A isolation ward, dedicated to the care of MHF patients, has been established by MSF.

11 April 2005: 231 cases (210 fatalities) now reported; Uige Province (202 cases, 190 fatalitie remains the most severely affected area. The present outbreak of MHF is unprecedented in its siz and urban nature, and the dimensions of its scope are still unfolding. Although surveillance to dete cases has improved, it remains patchy. In Uige, where mobile teams are active daily, surveillanc continues to concentrate largely on the investigation of deaths and the collection of bodies. Th security of teams remains a concern. More vehicles are needed and WHO is making the necessar arrangements on an urgent basis. To bring the outbreak under control, the detection and isolation patients needs to be made much earlier, but this will not happen until the public understands th disease and the high risks associated with treating patients in homes. Infection control needs improve in heath care settings, and WHO is continuing to supply protective equipment, for bo national and international staff, and appropriate for conditions in Africa. A welcome development the decision by the International Federation of the Red Cross and Red Crescent Societies strengthen its presence in Uige. Volunteers conduct a door-to-door public information and educati campaign, and is done in collaboration with community and church leaders and traditional healer Today, workers received specialized training from experts in social mobilization and medic anthropology drawn from the GOARN, and who have been deployed to deliver public talks markets and schools. These societies have extensive experience in responding to emergencies

Africa and have, in the past, been instrumental in bringing large outbreaks of Ebola under control. Because of this experience workers from the Federation are usually welcomed by communities. The WHO anticipates that this added and welcome support will create greater acceptance of control measures and help reduce high-risk behaviours.

Advice for travellers

Casual contact appears to play no role in the spread of MHF. Its transmission requires extremely close contact involving exposure to blood or other bodily fluids, and from a patient most likely showing visible signs of illness. The disease can be transmitted also following exposure to items, including bedding and clothing, recently contaminated by a patient. In addition, transmission can occur in hospitals that lack the equipment and training required for infection control. The hospital system in Angola has suffered from almost three decades of civil unrest, and several cases of MHF have occurred in health care staff exposed during the treatment of patients in Uige. To date, the WHO is not aware of Marburg occurring in foreign nationals other than those involved in the care of patients in Uige. The WHO does not recommend restrictions on travel to any destination within Angola, but does give precautionary advice:

- Travellers to Angola should be aware of the outbreak of MHF and of the need to avoid close contact with ill persons;
- Persons with existing medical conditions who might require hospitalization should consider deferring non-essential travel to Angola, particularly to Uige Province.
- Those travelling to Angola for the purpose of working in health care settings should be fully informed regarding the outbreak of MHF, should be equipped with effective personal protective equipment, and should be trained in the procedures to prevent transmission in such settings.
- Travellers leaving Angola are advised to seek medical attention should any illness with fever develop within 10 days of their departure; information about recent travel to Angola should be included when symptoms are reported.
- Health care workers and health authorities in countries neighbouring Angola should be aware of the outbreak and should be vigilant for cases.
- Countries having close ties with Angola, necessitating frequent travel there by their citizens, may want to consider the introduction of measures to increase vigilance for potential symptoms in persons returning from Angola; in some cases, the introduction of screening procedures to identify potentially infected persons may be considered.
- The WHO recommends that travellers with a clear exposure history be treated as contacts and placed under surveillance for 21 days, during which time their temperature should be monitored daily.

12 April 2005: 235 cases (215 fatalities) now reported; Uige Province remains the most severely affected area, with respective totals of 208 and 194. The isolation ward at the province's 400-bed hospital, especially equipped and staffed for the care of Marburg patients, stands empty, despite cases and known deaths in the community. It is apparent that, for the present, the community does not accept the concept of isolation. Residents are unwilling to report suspect cases and to allow them to be cared for under conditions reducing the risk of further transmission. Family members and others who refuse to allow patients to be cared for in the isolation facility are being informed how to protect themselves from infection and are given the appropriate supplies. Disinfectants, currently in short supply in Angola, are on urgent order by the WHO. Today, international staff drawn from the GOARN began training staff at the provincial hospital in the use of equipment and supplies to reduce the risk of infection in the health care setting. Fever-screening units are being established to ensure that all persons admitted to hospital are initially screened for symptoms of MHF before being admitted to the general wards. Apart from continuing security concerns, another pressing problem is poor access to remote communities in Uige Province and consequently poor surveillance for cases. Using a military helicopter, international workers have begun the pre-positioning of the supplies and

equipment needed for outbreak control in these areas, so that an immediate response can be launched should cases begin to occur.

In a tragic development, four Red Cross volunteers, freshly trained in social mobilization, were killed today by lightning while on their way to work. Support from Red Cross volunteers has been instrumental in controlling large outbreaks of the closely related Ebola haemorrhagic fever.

14 April 2005: 224 cases (207 fatalities) now reported, most have occurred in Uige municipality with respective figures of 175 and 163. Considerably fewer cases have been reported from a further municipalities in Uige Province. Improvement of public understanding of the disease and acceptance of control measures has become one of the most urgent priorities. Today, meetings were held in the WHO office in Uige with traditional community leaders - known as Sobas - from the entire Uige municipality. The governor of the province, and the director of health services, have released the Sobas from their current duties for seven days to accompany the surveillance and medical teams in their search for cases and to collect bodies. This is welcomed as an important step towards achieving community acceptance of measures needed to bring the outbreak under control. The WHO staff in Uige now plan to extend the same process, using locally respected Sobas, into all other municipalities affected by the disease, and is supported by the authorities. Training to protect staff at the provincial hospital from infection, and to reduce the risk of transmission, is continuing. Also, 82 nurses in high-risk departments, including the maternity ward and the laboratory, were trained. Heads of departments and doctors were trained yesterday; training for health staff in private clinics, and for health workers in the police force, is planned for early next week.

In response to an international appeal launched on 8 April, the WHO has received pledges of funding from Germany, Sweden, the Netherlands, the United Kingdom, and the European Commission Humanitarian Office (ECHO).

19 April 2005: 266 cases (239 fatalities) now reported, in Uige Province, which remains the epicentre of the outbreak, the respective totals are 250 and 228. A team of 28 Angolese health care professionals, assembled by the government, arrived today in Uige to provide further support to control activities. They have been assigned to work on infection control, surveillance for new cases and the tracing and management of contacts, and are being trained and equipped for these tasks. The new staff will also join teams visiting communities within the affected municipalities in order to improve public understanding of the disease and acceptance of control measures. These teams are already being supported by Sobas, and local volunteers. Public understanding of the disease, and participation in the outbreak response, remain the most important factors influencing successful control. Activities needed to prevent further transmission within the hospital setting are well under way. In addition, protective gloves, soap, and instructions about the importance of their use have been provided to traditional healers and midwives. Significant progress has been made in increasing the engagement of affected communities. Some cases and deaths have, however, continued to occur within the community. Efforts to rapidly isolate cases, shortly after symptom onset, and to follow contacts, need to be intensified further. Teams investigating recent deaths within the community have determined that some families – while providing care in their homes - are administering injections to patients, a high-risk practice which can perpetuate the transmission of Marburg within the community. Educational messages and materials communicating the associated dangers were developed today and will be added to the information already being provided to communities.

Second assessment of the outbreak: The international response to the outbreak in Angola began one month ago (22 March). The features of MHF, and the conditions in Angola, have been an extreme test for the capacity of international community to hold emerging diseases at bay. The outbreak in Angola is the largest and deadliest on record for this rare disease, with a case fatality rate above 90%; outbreaks of the closely related Ebola haemorrhagic fever have mortality rates ranging from 53% to 88%. The only other large outbreak of Marburg, in the Democratic Republic of Congo

(1998-2000), had a case fatality rate of 83%. Two factors make the rapid detection of outbreaks of Marburg haemorrhagic fever difficult:

- the extreme rarity of this disease, and
- its similarity to other diseases seen in countries where deaths from infectious diseases are common.

Neither the source nor the date of the initial cases in Angola can at present be identified. Experience with outbreaks of other VHF (including Ebola) indicates that outbreaks of Marburg can be ended using classic public health interventions. In theory, the measures required in Angola are both few and straightforward:

- Rapid detection and isolation of patients;
- tracing and management of their close contacts;
- infection control in hospitals - and protective clothing for staff - to disrupt the chain of transmission and thereby seal off opportunities for the further spread of Marburg disease.

Such straightforward measures are, however, complicated by the distinct features of this disease (sudden onset, dramatic symptoms, rapid deterioration of patients) and the absence of a vaccine and effective treatment, invariably instil great anxiety in affected populations. This anxiety can hamper control operations, especially when communities begin to conceal cases (and bodies) because of their suspicions around the 'safety' of hospitals. These suspicions are understandable because very few patients with laboratory-confirmed MHF, survive; most hospitalized patients die within a day or two following admission. For affected communities, staff from the mobile teams, fully suited in protective gear, are seen to take away loved ones seldom to be seen again alive. The WHO staff in Uige today reported that community attitudes are improving, although hostility towards the mobile teams is of concern in one area with recent cases and deaths. Efforts to sensitize affected communities continue, with local volunteers supported by Portuguese-speaking experts from Brazil and Mozambique. Conditions in Angola - weakened by almost three decades of civil unrest – present additional challenges. Supplies of water and electricity are intermittent, even in health care facilities; the weakened communications and transportation infrastructures, are an added problem. Yesterday, the WHO office in Uige was informed of a death in another municipality, but was unable to collect the body because of poor roads. Fortunately, the spread of the disease beyond Uige Province, located in the interior of the country, has been limited.

The WHO believes that the risk for the international spread of Marburg is low. No foreign nationals, with the exception of those involved in the direct care of patients, have been infected. There is no evidence that people can spread the virus before the onset of symptoms. Shortly after symptom onset, patients become rapidly, and visibly, very ill. The WHO is optimistic that the outbreak can be controlled if present activities continue with sufficient vigour. All the essential containment measures are being applied with extensive international support, including more than 60 international staff drawn from institutions in the GOARN, and the cooperation of national authorities and experts. Tools and methods developed during international responses to outbreaks of other diseases have all been brought to bear on the present outbreak, and the success of this collaborative effort has surpassed initial expectations. Needs, which have ranged from satellite telephones and hand-held radio sets to vehicles, protective equipment, disinfectants, and specialized staff, have been rapidly communicated, and immediately met. An important present goal is to transfer skills and responsibilities for outbreak response to national staff, and, with this goal in mind, training has commenced. However, the WHO and its partners are nonetheless prepared and organized to continue the outbreak response for several additional months, if necessary.

27 April 2005: 275 cases (255 fatalities) now reported; in Uige Province, which remains the epicentre of the outbreak, the respective totals are 266 and 246. With all control measures – teams, equipment, and protocols – now in place, extreme care must be taken to guard against any practices that could again amplify transmission. At this point, an amplification event would set back the presently intense containment efforts by several weeks. In past outbreaks of VHF, such events

resulted in two additional transmission cycles, and a second wave of cases. Control operations in Uige have experienced some recent setbacks.

- Earlier this week, doctors at Uige's large provincial hospital were twice directly exposed to the blood of patients being treated in general wards, without adequate infection control. The doctors are under observation but these high-risk exposures should never have occurred indicating that infection control procedures in the hospital have been seriously compromised. They occurred despite an operational safety system being in place, to screen new admissions for a prior exposure history and for fever, and to ensure the separation of possible cases from patients already in the general wards.
- In another incident, the body of a deceased patient was left, uncleaned, in an open ward for more than eight hours, placing hospital staff and other patients at risk.
- In a third incident, a severely ill baby admitted to the paediatric ward was placed in a cot, without disinfection, immediately after the body of another baby, who had died from the disease, had been removed. Furthermore, and in line with cultural practices, mothers are present in the paediatric ward and share in the care of severely ill children, thus also sharing exposure risk.

Under such conditions, the amplification of transmission is highly likely to occur. Had safety protocols, set in place by the international team, been followed, none of these incidents would have occurred. Closing the hospital was not considered a viable option. Such a step would deprive many patients of potentially life-saving care while re-directing others to private clinics, where conditions and practices are even less safe, thereby increasing the likelihood of additional cases. Yesterday, the Minister of Health, accompanied by a vice-minister and the head of the WHO office in Angola, flew to Uige to investigate the situation, to find solutions, and to oversee their implementation. The officials have recognized that strong measures are needed to ensure that patients admitted for other conditions do not risk Marburg infection. The first corrective steps were put in place, and involved the collaboration of ministry officials, the WHO, and MSF; the WHO has decided to strengthen also the presence in Uige of international staff specialized in infection control and has welcomed the direct intervention of ministry officials. This high-level support should help ensure that containment measures, previously set in place and of proven efficacy, are restored and fully adhered to. The investigation of several recent fatalities in Uige indicates a clear link between home-based treatments using unsafe syringes and the spread of Marburg virus. This problem is being addressed urgently. A massive door-to-door campaign, supported by banners and posters throughout Uige municipality, was launched yesterday to inform residents of the associated dangers and to collect and safely destroy syringes. The campaign continued today.

3 May 2005: 308 cases (277 fatalities) now reported; in Uige Province, which remains the epicentre of the outbreak, the respective totals are 297 and 266. The large increase in the number of reported cases for Uige is the result of retrospective investigations adding cases to the database. However, new confirmed cases, and deaths continue to be reported in Uige. Limiting opportunities for spread by the virus is essential. Procedures and assigned responsibilities for safe infection control at the provincial hospital in Uige have been agreed on this week by ministry officials, the WHO, and MSF. Teams are giving particular attention to screening and admission procedures to prevent suspected cases from being treated in open wards. To support these efforts, the WHO has deployed additional experts in infection control drawn from institutions in the GOARN. Massive public information campaigns aimed at ending unsafe injections continued this week. Five new vehicles have been provided by the Angolan government providing greater mobility to investigate suspect cases and deaths, and to follow contacts.

10 May 2005: 316 cases (276 fatalities) now reported; the municipality of Uige remains the most severely affected in the province, and where new cases have been identified in the last few days. As some chains of transmission are still ongoing, mobile teams are investigating suspect cases and

following contacts. Religious leaders have joined the information campaign against the use of unsafe injections.

17 May 2005: 337 cases (311 fatalities) now reported; the vast majority have occurred in Uige Province, where the respective totals are 326 and 300. No cases have been reported outside Uige for the past five weeks. Infrastructures and protocols for controlling the outbreak are in place and functioning well. The isolation unit at Uige's provincial hospital is being used, infection control in the hospital has improved, and safe burial practices are now being followed. Portable field laboratories continue to provide rapid diagnostic support. A campaign to stop home treatment of patients using unsafe injections has resulted in the collection and disposal of a large number of needles and syringes. The campaign, which has been supported by religious and community leaders and volunteers from the local Red Cross, is thought to have raised public awareness considerably. Support from religious and community leaders has also allowed the mobile surveillance teams to operate more smoothly, increasing the efficiency of case finding and contact tracing. However, new cases, linked to exposure in homes and at funerals, indicate that public understanding of the disease needs still to be improved.

26 May 2005: 399 cases (335 fatalities) now reported; the vast majority have occurred in Uige Province where the respective totals are 388 and 324. Yesterday, four new suspected cases (three fatal) were reported from Bungo municipality, Uige Province; two have been confirmed and are the first to be detected in Bungo since early April. An urgent investigation has been launched to determine whether they can be linked to Uige municipality, where transmission is ongoing. Another focus of transmission would be a disturbing development. This week, the mobile surveillance teams were able to visit more than half of the 100 persons known to have had close contact with a Marburg patient. New cases are, however, continuing to occur with no known link to a previous case, suggesting that the surveillance system has not yet reached the efficiency needed to interrupt chains of transmission. Local and international staff have continued to identify cultural practices that create opportunities for exposure to the virus thus allowing the outbreak to continue. Most recently, around 200 traditional healers have been trained in ways to reduce risks to themselves and their clients and were given masks and gloves. To date, at least two traditional healers have died of MHF. Intensive educational campaigns, supported by local religious leaders and Red Cross volunteers, about the hazards of home treatment using injections, have resulted in the collection and disposal of large numbers of syringes. It is not certain whether this practice - highly efficient for spreading the virus - has been fully eliminated in Uige's population.

5 June 2005: 423 cases (357 fatalities) now reported; the vast majority occurred in Uige Province, where the respective totals are 412 and 346. The number of new cases being reported in Uige municipality has declined considerably, with only 1 new confirmed case detected in the past week: a recognized contact under follow up. In comparison, during the peak of the outbreak (late March to April) 30 to 40 new cases were being reported weekly. Alerts to potential cases continue to be received and investigated, indicating that vigilance remains high.

16 June 2005: 422 cases (356 fatalities) now reported; 21 contacts are being followed in Uige municipality and 111 in other municipalities. Alerts to potential cases in difficult to reach locations outside of Uige have been received and it is planned for the team to travel by helicopter to investigate them.

10 July 2005: Following the review of data by the Outbreak Response Team, the Ministry of Health has reported a total of 351 cases and 312 deaths from MHF; 64 contacts are being followed up in Uige Province. The team continues to receive and investigate alerts to potential cases. Clinical specimens from alerts are being transported to the Public Health Agency of Canada's National Microbiology Laboratory. Continuing support for infection control will be provided to hospitals and health centres in Uige Province.

CONCLUSIONS – OLD LESSONS AND NEW

1. In Angola, contingency plans for VHF were not well established and/or up-dated both at national and provincial levels prior to the outbreak, which might significantly have contributed to early recognition of VHF cases and rapid implementation of adequate barrier nursing and other hospital/outbreak infectious control measures.
2. Cases of VHF are often first suspected or diagnosed in African general hospitals which often lack basic equipment and supplies, designated facilities for isolation of VHF cases, and staff which is experienced in recognition and barrier nursing of VHF patients.
3. In many African countries, surveillance for communicable diseases is hampered by constraints in communications, unreliable electricity supplies, limited transport, etc. The WHO Polio Eradication Programme provides key technical and operational support for all such surveillance programs, but is focused primarily on events relating to polio and measles and not for detecting and reporting on other unusual disease events. Nevertheless, it played an instrumental role in initial recognition of the current Marburg outbreak in Angola.
4. There was insufficient capacity amongst provincial health care staff in Angola to adequately evaluate the reports of suspected cases of MHF, also, the Ministry of Health was not able to provide guidelines and tools in disease-affected areas timeously.
5. Knowledge, and practice, of safety standards, and precautions for infection control, were lacking.
6. Protective equipment for personnel, and other materials required for standard precautions in the health care facilities, were inadequate.
7. The distribution of personal protective equipment was wasted in the absence of appropriate guidelines and training in their use.
8. The local health teams were well-intentioned, professional and motivated, but lacked the knowledge and skills for the tasks they had to undertake.
9. The ability of international teams to make effective interventions was hampered by severe language barriers.
10. Laboratory confirmation of suspected Marburg cases, and especially at the beginning of the outbreak, was often not possible due to unavailability of clinical specimens or their poor quality.
11. High level of sensitivity surrounding collection of blood specimens from suspected Marburg cases often prevented the use of invasive sampling methods, necessitating nasal and oral swabs. Although, these clinical specimens excluded the use of ELISA-based assays, they proved to be useful for rapid and accurate PCR-based diagnosis of MHF.
12. The international support and response to the outbreak was instrumental in bringing it under control in Uige Province, preventing its wider spread within the country and possibly outside Angolan boundaries.

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AN EVALUATION OF A WEST NILE VIRUS OUTBREAK IN HORSES: 2002

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SUMMARY

The objective of this study was to characterize an outbreak of West Nile virus (WNV) infection in horses in North Dakota in 2002, evaluate vaccine effectiveness, and determine horse characteristics and clinical signs associated with infection. Affected horses (569) were distributed in 52 out of 53 counties of North Dakota, ranging from 1 to 50 horses/county, and they occurred from June to October, 2002, peaking in August. The horses exhibited various clinical signs characteristic of encephalomyelitis associated with WNV infection. Among horse cases, 27% (152) were vaccinated against WNV, 54% (309) were not, and 19% (108) were of unknown status. Also, 61% (345) of the horses recovered, 22% (126) died, and the outcome of 17% (98) was unknown. The odds of death among unvaccinated horse cases were 3 times (OR = 3.2, 95% CI = 1.5 – 6.8) and 16 times (OR = 16.1, 95% CI = 1.7 – 147.9) more than among those that received only one dose or 2 doses of vaccine not given per the manufacturer's recommendation, and those that were vaccinated as per manufacturer's recommendations, respectively. Horses exhibiting recumbency, posterior paresis and increased age were associated more strongly with death, whereas those with incoordination were associated with lower odds of death.

The results of this study show how horses vaccinated with WNV vaccine, especially as per manufacturer's recommendations, were more likely to survive the infection compared to those that were not. Also, clinical signs and horse characteristics could be used to predict the outcome to WNV infection with horses showing incoordination being more likely to survive while older horses, and those that were recumbent and / or showed posterior paresis were more likely to die compared to those without these symptoms

INTRODUCTION

West Nile virus (WNV) is an arbovirus of the genus Flavivirus, family Flaviviridae. The virus was first isolated from a febrile woman in Uganda in 1937 in the district of West Nile (Hayes 2001). Since then, WNV encephalomyelitis has been reported in humans, birds and horses in Africa, Europe, the middle East, West and Central Asia, and Oceania. WNV was first reported in the United States in 1999 in New York (Trock, Meade, Glaser, Ostlund, Lanciotti, Cropp, Kulasekera, Kramer & Komar 2001), where it caused disease in birds, horses and humans. Since then, WNV has spread across the country. In 2002, there were more than 15,257 laboratory-confirmed cases of WNV infection or seroconversion in horses reported in 43 states (USDA 2003). Spread by mosquitoes (Turell, Sardelis, Dohm, & O'Guinn 2002), WNV mainly causes disease in birds, equids (horses, donkeys, mules, and ponies), and humans (CDC 2002). The 2002 WNV epidemic in the United States was the largest arboviral meningoencephalitis epidemic reported in the western hemisphere (CDC 2002). The epidemic was most intense in the central United States, with the region's highest numbers of equine cases (700 to 1,100 cases/state) in Illinois, Indiana, Iowa, Kansas, Missouri, and Nebraska (USDA 2003). In North Dakota, WNV infection was first reported in 2002, and 569 equine cases were detected (Schuler, Khaitsa, Dyer & Stoltenow 2003). There was no evidence of WNV in North Dakota in 2001 (Schuler, Khaitsa, Dyer & Stoltenow 2003). West Nile virus was detected in birds in Wisconsin and Iowa in late 2001, and neither Wisconsin nor Iowa had any equine cases in 2001. Active surveillance of WNV in birds in North Dakota began in June 2002, and WNV was identified in North Dakota approximately 2 to 3 weeks

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before it was identified in Minnesota, the adjacent state to the east. In 2002, Canadian health authorities also reported WNV in 5 provinces (Health Canada 2002).

A few detailed reports (Salazar, Traub-Dargatz., Morley, Wilmot, Steffen, Cunningham & Salman 2004) were made of WNV encephalomyelitis as it first occurred in immunologically naïve horses in the United States. Data suggest that approximately 40% of infections result in death of the horse and that most horses recover from the infection (Salazar, Traub-Dargatz., Morley, Wilmot, Steffen, Cunningham & Salman 2004). A WNV vaccine provisionally licensed by the USDA first became available for use in horses in August 2001 and is now used to prevent viremia in horses exposed to WNV (USDA 2003). However, the effectiveness of this WNV vaccine in protecting horses in an epidemic was unknown. The manufacturer recommends that adult horses receive 2 vaccinations 3 to 6 weeks apart, foals receive 3 vaccinations during the same interval, and the full series be completed prior to the vector (mosquito) season (USDA 2003). Annual boosters are recommended prior to the mosquito season (USDA 2003). The objective of the study reported here was to describe the initial North Dakota outbreak of WNV infection in horses in 2002 determine the characteristics of the disease, estimate the effectiveness of the available vaccine, and determine which horse characteristics and clinical signs were associated with outcome.

MATERIALS AND METHODS

Criteria for Selection of Cases

A horse with WNV infection was defined as a horse with typical clinical signs and seroconversion against WNV, as measured by use of IgM capture ELISA.

Procedures

Data for affected horses were retrieved from medical records for 2002 obtained from the North Dakota State University Veterinary Diagnostic Laboratory (VDL). Diagnosis of WNV infection in all horses was performed by the same staff at VDL, thus minimizing diagnostic bias. Additional data were obtained by use of a questionnaire mailed to the veterinarians who treated the horses.

Laboratory methods

Specimens (serum, blood, CSF, brain, and spinal cord tissue) from horses with clinical signs and suspected of having WNV infection were submitted to VDL from veterinarians across the state. No laboratory fees were charged, which may have been an incentive for submission of samples. An immunoglobulin IgM capture ELISA developed by the USDA Animal and Plant Health Inspection Service (Ostlund, Crom, Pederson D, Johnson, Williams, & Schmitt 2001) and modeled after an EEE MAC-ELISA (Sahu, Alstad, Pedersen, & Pearson 1994) was used to detect seroconversion. Confirmatory diagnosis obtained from specimens from the initial 85 horses in this study was accomplished by use of virus isolation, PCR assay, or Plaque-reduction neutralization tests performed at National Veterinary Services Laboratory (NVSL) in Ames, Iowa. In addition, NVSL also performed IgM capture ELISA tests on the serum samples. There was 100% agreement between the VDL and NVSL results. Confirmatory testing at NVSL was discontinued at NVSL's request because of the excessive national demand for testing.

Statistical analyses

Descriptive statistics of horses that tested positive for WNV infection were computed by the help of SAS software (*SAS/STAT user's guide*: version 8. Cary, NC: SAS Institute Inc, 1999;298–299). Characteristics of vaccinated and nonvaccinated horses were compared by use of χ^2 tests of independence. Software (Geographic Information Systems, ArcInfo 8, ESRI, Redlands, Calif.) was used to display the spatial distribution of all horses and infected horses in the state by county. Because of difficulty resulting from the large extent of the study area and temporal and resource restrictions, geocoding of individual addresses was not done on the available data to determine specific coordinates for locations of infected horses. Administrative base maps were obtained (ESRI

Data and Maps, 1999 edition, ESRI, Redlands, Calif.) and the main thematic data layers were assembled by use of a software program (ArcInfo 8, ESRI, Redlands, Calif.). The association between time-since-vaccination and outcome was investigated. Time-since-vaccination was computed as time in days from the last vaccination to onset of clinical signs or time when the veterinarian was contacted.

Logistic regression analysis as described by Hosmer & Lemeshow (2000) was used to identify horse characteristics and clinical signs that were associated with outcome. Outcome (death) was used as the dependent variable. Independent variables included 10 clinical signs (present vs absent) and certain demographic variables, including age (≤ 5 years vs > 5 years), sex (male vs female), breed (Quarter Horse vs other), horse type (stock vs other), and vaccination history (no vaccination, 1 vaccination or 2 vaccinations but not following manufacturer's recommendations, or 2 vaccinations following manufacturer's recommendations). Several selection methods were evaluated along with an assessment of goodness-of-fit statistics (as described by Hosmer & Lemeshow, 2000), and the stability of the parameter estimates during the modeling process to determine the final multiple logistic regression model. Vaccination history was incorporated into the model with a reference cell approach, with the nonvaccination group as the reference group. Collinearity among the independent variables (especially the clinical signs) was evaluated and not problematic. Simple Pearson correlation coefficients among the clinical signs were low ($r < 0.25$), which supported the collinearity results. For all final comparisons, a value of $P < 0.05$ was considered significant.

RESULTS

In 2002, specimens from 1,028 horses were submitted to the VDL. Of these, 769 (75%) were from horses that met the case definition for this study. Of the 769 cases, 569 were from North Dakota and were included in the study. Questionnaires were mailed to veterinarians of the 569 affected horses, and all questionnaires were returned. Of the 569 horses, 345 (61%) recovered, 126 (22%) died, and 98 (17%) had an unknown outcome. Among horses that died, 77 (61%) were euthanatized and 49 (39%) died from disease. Also, 152 of the 569 (27%) horses were vaccinated, 309 (54%) were not vaccinated, and 108 (19%) had unknown vaccination history.

Cases of WNV infection in horses were reported from 52 of the 53 counties of North Dakota (no affected horses were reported from Divide County), and the number of affected horses ranged from 1 to 50, with the most reported from the central and southeastern counties (Figure 1). Counties with the highest incidence and counties with the highest number of horses did not necessarily match. After accounting for the total number of horses at risk per county, the incidence of equine cases (number of equine cases/1,000 horses) was higher in the eastern and northeastern parts of the state. Ninety-eight veterinarians participated in the study, and the median number of cases per veterinarian was 5 (range, 1 to 18; mean, 5.8). The majority (84%) of the veterinarians evaluated 1 to 10 affected horses; only 16% evaluated more than 10 affected horses. Affected horses had a range of clinical signs that were mainly attributable to encephalomyelitis, with incoordination (69%) reported most frequently (Table 1).

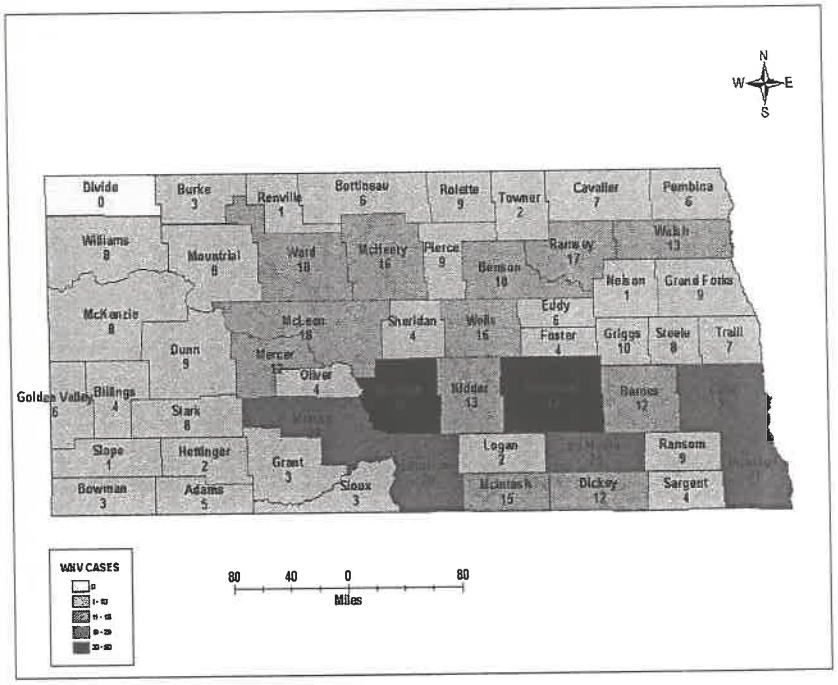

Figure 1. Geographic distribution (by county) of 569 horses with West Nile virus (WNV) infection or exposure in North Dakota, 2002

Table 1. Distribution (No. [%]) of horses with West Nile virus infection in which various clinical signs were evident

Clinical sign	No. (%) horses
Incoordination	330 (69)
Muscle tremors, twitching face or muzzle	248 (52)
Weakness or paralysis of limbs	182 (38)
Caudal paresis	141 (29)
Recumbency, difficulty rising, or both	111 (23)
Lip droop	101 (21)
Teeth grinding	40 (8)
Fever	36 (7)
Circling	29 (6)
Blindness	16 (3)

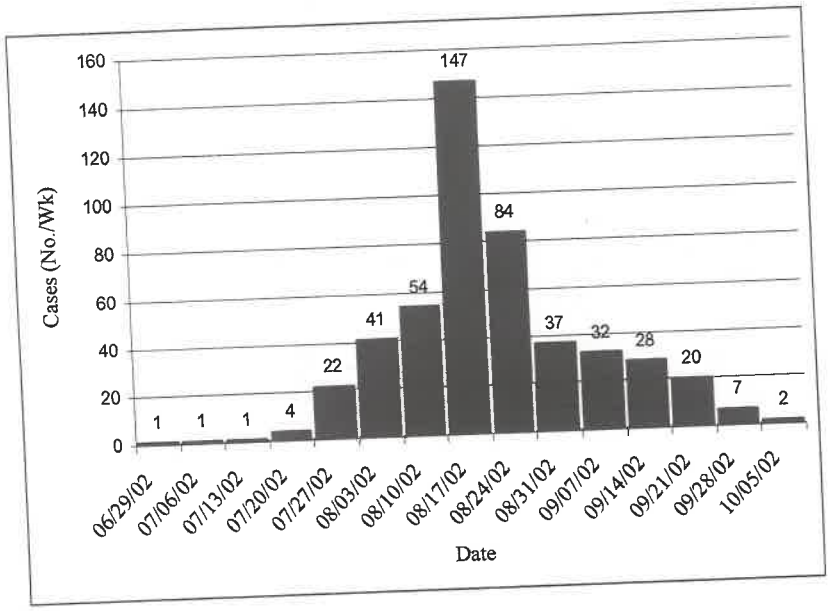

Figure 2. Temporal distribution of 481 horses with WNV infection or exposure in North Dakota, 2002

Cases were reported from June to October 2002 (Figure 2), with the majority (363/481 [76%]) occurring in August 2002. Recovery times varied from 1 day to 3 weeks with the majority (94%) ranging from 1 to 14 days (median, 7.2 days). The majority of horses (334/569 [59%]) were Quarter Horses, with at least 15 horse breeds reported, and the predominant horse type was stock (352/420 [85%]); the remainder were light breeds (8%), draft horses (5%), and ponies (2%). Age of affected horses ranged from 3 months to 30 years, with a median of 8 years. Age-specific case fatality rate was skewed toward older horses (Figure 3). Of the 152 vaccinated horses, data on time-since-vaccination were available for 113 (72%) of the horses. For horses that died from disease (n = 7), this mean value was 5.4 days (median, 7 days; range, 1 to 9 days); for horses that were euthanatized, this mean value was 15.7 days (median, 7 days; range, 1 to 67 days); and for horses that recovered (n = 94), this mean value was 10.7 days (median, 12 days; range, 0 to 44 days). There was a significant ($P = 0.049$) association between time-since-vaccination and outcome of dying, being euthanized, or recovering. Horse cases with a relatively shorter time-since-vaccination interval were more likely to die or be euthanatized than those cases with a longer time-since-vaccination interval.

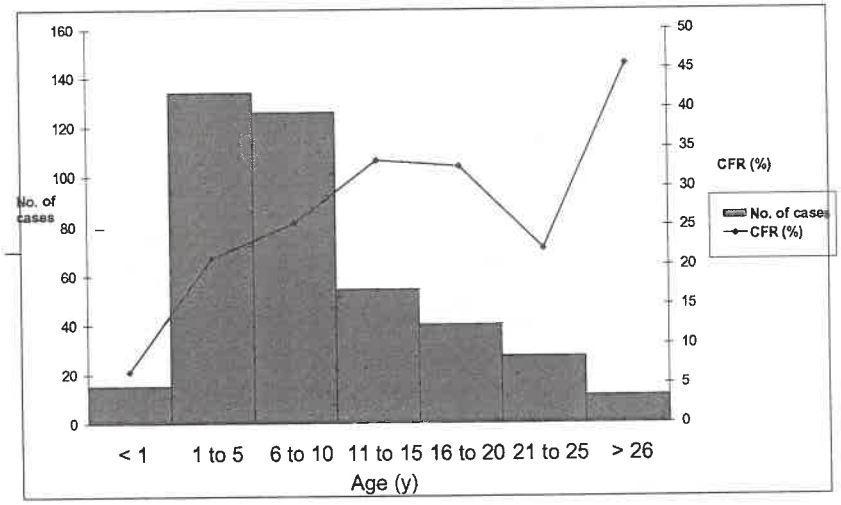

Figure 3. Age distribution (bars; n = 569) and corresponding case fatality rate (CFR; diamonds; n = 471) of horses with WNV infection in North Dakota, 2002

The case fatality rate among horses that were vaccinated (22/152 [14%]), compared with those that were not vaccinated (104/309 [34%]), was found to be significantly different ($P < 0.001$) by use of the χ^2 test. A significant ($P < 0.01$) difference in case fatality rate was detected between horses that were not vaccinated (case fatality rate, 34%) and those that were vaccinated with only 1 dose or 2 doses that were not administered as per manufacturer's recommendations (case fatality rate, 16%). Also, a significant ($P < 0.01$) difference in case fatality rate was detected between horses that were not vaccinated (case fatality rate, 34%) and those that received 2 doses administered according to manufacturer's recommendations (4%). However, the difference in case fatality rate between horses that received vaccination as per manufacturer's instructions and those that received vaccination but not per manufacturer's instructions was not significant. A Bonferonni adjustment was made to the pairwise comparisons of case fatality rate among the 3 vaccination categories to correct for multiple testing.

A significant difference was also detected in outcome to WNV infection (death or recovery), depending on whether horses had specific clinical signs. Recumbency, caudal paresis, teeth grinding, and weakness or paralysis were associated with increased odds of death, whereas incoordination and lip droop were associated with decreased odds of death, compared with affected horses that did not have these characteristics (Table 2).

Vaccinated versus nonvaccinated horses differed significantly only in regard to proportion with recumbency ($P = 0.018$) and lip droop ($P = 0.017$). Fewer vaccinated horses were recumbent and more vaccinated horses had lip droop, compared with nonvaccinated horses.

Table 2. Univariate analysis of associations between death rate and various clinical signs, characteristics, and vaccination history variables among 471 horses with West Nile virus infection

Variable	Category	N	Dead (%)	*P* value
Recumbency*	Present	110	79	0.001
Incoordination†	Present	318	18	0.001
Caudal paresis*	Present	139	41	0.001
Lip droop†	Present	100	15	0.002
Weakness or paralysis*	Present	178	34	0.003
Teeth grinding*	Present	39	44	0.016
Horse type	Stock Other*	352 68	24 41	0.004
Breed*	QH Other*	334 111	23 37	0.006
Age (y)	> 5 ≤ 5	153 245	23 31	0.076
Sex	Male Female	217 210	24 32	0.085
Vaccination history	None* CM + NCM	309 152	33 14	0.001
Vaccination regimen	None* NCM	309 127	33 16	0.001
Vaccination regimen	None* CM	309 25	33 4	0.016
Vaccination regimen	NCM CM	127 25	16 4	0.150

N = No. of horses. *Variables significantly ($P < 0.05$) associated with higher death rate, compared with horses without those characteristics. †Variables significantly ($P < 0.05$) associated with lower death rate, compared with horses without those characteristics. CM = Vaccination in compliance with manufacturer's recommendations. NCM = Vaccination not in compliance with manufacturer's recommendations.

The final multivariate logistic regression model included the variables incoordination, caudal paresis, recumbency, age, and vaccination history. Horses with caudal paresis (OR, 2.6; 95% CI, 1.4 to 5.1), recumbency (OR, 27.2; 95% CI, 13.9 to 53.2), and age > 5 years (OR, 2.3; 95% CI, 1.2 to 4.4) were more likely to die than those without these signs or characteristics. Horses with incoordination (OR, 0.3; 95% CI, 0.1 to 0.5) were less likely to die than those without this clinical sign. Horses that received 1 or 2 doses of vaccine that were not given per the manufacturer's recommendation (OR, 0.32; 95% CI, 0.15 to 0.68) and those that were vaccinated as per manufacturer's recommendations (OR, 0.062; 95% CI, 0.007 to 0.58) were less likely to die than those that were not vaccinated. The sample size available for the final logistic regression model was 389, concordance was 88.8%, actual misclassification rate was 11.8%, and the Hosmer & Lemeshow (2000) goodness-of-fit test suggested the model was adequate ($P = 0.748$).

DISCUSSION

In this WNV epidemic, 73% (345/471 horses with known outcome) of horses with WNV infection recovered from the disease. This agrees with data in the literature (Ostlund, Andresen & Andresen 2000; Ward, Levy, Thacker, Ash, Norman, Moore & Webb 2004) that suggest that most affected horses recover. Also, the percentage of horses with muscle tremors or fasciculations (52%), lip droop (21%), teeth grinding (8%), and blindness (3%) were similar to that of horses reported with WNV infection in 2000 from 33 states (Ostlund, Crom, Pederson D, Johnson, Williams & Schmitt 2001).The clinical signs we observed shared similarities with those reported in other epidemics (Durand, Chevalier, Pouillot, Labie, Marendat, Murgue, Zeller & Zientara 2002; Autorino, Battisti, Deubel, Ferrari, Forletta, Giovannini, Lelli, Murri & Scicluna 2002). It is possible that the number of horses reported as dead or even those that recovered was underestimated, given that our sample included only specimens or horses that were tested at VDL. Also, the outcome of a fairly large proportion (18%) of the affected horses was unknown. Increasing age was generally associated with higher case fatality rate, although this was not true for horses that were 21 to 25 years old.

The wide age range (4 months to 38 years) was similar to that reported in 33 other states (Ostlund, Crom, Pederson D, Johnson, Williams & Schmitt 2001), and elsewhere (Autorino, Battisti, Deubel, Ferrari, Forletta, Giovannini, Lelli, Murri & Scicluna 2002), suggesting lack of previous exposure to WNV in the horses. Clinically affected horses and those that died in the US outbreaks of 1999 and 2000 were generally older than reported here (median age 14.5 years in 1999, 16 years in 2000) (Ostlund, Crom, Pederson D, Johnson, Williams & Schmitt 2001). Others (Snook, Hyman, Del Piero, Palmer, Ostlund, Barr, Desrochers & Reilly 2001) reported a median age of 8 years, similar to ours, for horses that were euthanized.

A variety of horse breeds have been affected by WNV elsewhere (Ostlund, Crom, Pederson D, Johnson, Williams & Schmitt 2001), which is similar to our findings. The spatial distribution of cases in North Dakota matched closely that of known areas of concentration of migratory birds in the state. For several reasons, migratory birds have long been suspected as the principal hosts for introduction of WNV into new regions. Outbreaks of the virus in temperate regions generally occur during late summer or early fall. There may be a temporal relationship between the spring hatching of migratory birds and subsequent increase in the vector population. These outbreaks also occur in humans living in or near wetlands where high concentrations of birds come into contact with large numbers of mosquitoes, and antibodies to WNV virus and the virus itself have been detected in some species of actively migrating birds ((Rappole, Derrickson & Hubalek 2000). In 2002, seropositive wild-caught birds were reported from 21 North Dakota counties (North Dakota WNV), which included 5 of the 7 counties with most equine cases (≥ 19), and the counties with most cases were located across the migratory routes for wild birds (USGS breeding bird survey). Elsewhere in the United States in 2002, 144 seropositive wild-caught birds and 366 seropositive captive sentinel birds were reported from 72 counties in 11 states (Florida, Indiana, Iowa, Kansas, Louisiana, Nebraska, New York, North Carolina, Ohio, Pennsylvania, and Texas) (CDC 2002) A possible association between case sites and proximity to communal bird roosts or waterfowl congregations was reported in a case-control survey conducted by the USDA in 2001.

The highest incidence of equine cases was in the eastern and northeastern parts of North Dakota, which is the wet region of the state (The National Water Conditions Map) It is possible that the eastern part of the state provided better conditions for mosquitoes to breed, compared with other parts of the state, thus creating favorable conditions for WNV transmission to horses. Because most WNV infections in horses do not cause disease, all infected horses may not have been identified.

The temporal distribution of equine cases in North Dakota in 2002 matched that of cases from the 10 states from which data were available (CDC 2002), with onset of illness from June or July to November and peaking in August or September. In North Dakota in 2002, equine cases preceded avian cases. It is possible that avian cases preceded equine cases, as has been reported elsewhere (CDC 2002) but were not observed earlier because passive bird surveillance in the state started in

June. The 17 human cases (2 deaths) that were reported during 2002 in North Dakota occurred simultaneously with the equine cases, as was reported in other midwestern and north-central states (CDC 2002), although the human cases occurred somewhat later in the season than the equine ones. Nationally, the epidemic peak of human WNV-associated illness during 2002 occurred in late August (CDC 2002); human cases in southern states preceded those in northern states by approximately 1 month. In New York in 1999 and 2000, it was reported that equine cases occurred after human cases had been identified (Trock, Meade, Glaser, Ostlund, Lanciotti, Cropp, Kulasekera, Kramer & Komar 2001). In North Dakota, it is possible that aggressive vector control and public education efforts by state and local public health officials limited the number of human cases. A study (Ruiz, Tedesco, McTighe, Austin & Kitron 2004), reported that differential mosquito abatement efforts were especially important risk factors to occurrence of WNV infection in humans.

Fever was reported in only 6% of our affected horses, an indication that the viremia in horses may be short-lived or that many horses do not develop clinical signs, as has been reported (Ward, Levy, Thacker, Ash, Norman, Moore & Webb 2004). A transient, low-level viremia has been detected in naturally and experimentally infected horses and donkeys (Schmidt & Mansoury 1963). Research suggests that birds are the only animals in which a viremia develops that is sufficient to infect mosquitoes and propagate the infective cycle (Rappole, Derrickson & Hubalek 2000).

Significantly fewer vaccinated horses had recumbency, compared with nonvaccinated horses; therefore, vaccination may have reduced the odds of death, because recumbency was the clinical sign most strongly associated with death. Interestingly, horses that had incoordination and lip droop were less likely to die than those that did not. It is possible that horses with incoordination and lip droop were in a stage of recovery, and had remained alive long enough for these clinical signs to be noticed by the veterinarian and for them to receive supportive treatment, which further improved their chances of survival. Horses that did not have these clinical signs possibly died suddenly or progressed to recumbency quickly and were euthanatized as a result.

In other studies (Salazar, Traub-Dargatz, Morley, Wilmot, Steffen, Cunningham & Salman 2004), it was also reported that lower odds of death were evident for horses that were vaccinated, compared with those that were not. In our study, even horses that received only 1 or 2 doses of vaccine not given as per manufacturer's recommendations had lower odds of death than nonvaccinated horses, suggesting that any vaccination is beneficial to survival. Horses vaccinated according to manufacturer's recommendations had even lower odds of death than those that were not vaccinated which further supports the beneficial effect of vaccination.

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IDENTIFICATION OF RODENT SPECIES THAT PLAY A ROLE IN DISEASE TRANSMISSION TO HUMANS IN SOUTH AFRICA

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SUMMARY

Rodents of the family Muridae play an important role as wildlife reservoirs of zoonotic infectious diseases. Many zoonotic pathogens that are endemic and avirulent in murid rodents are virulent in humans. These may be transmitted either directly or indirectly and include bubonic plague, leptospirosis, and toxoplasmosis. In order to manage pests and their diseases effectively, it is critical that the murid rodents be identified to the species level. In South Africa, where approximately 50 murid rodent species are known to occur, traditional morphology-based methods are not always able to distinguish between species. One case in point is *Mastomys natalensis* and *M. coucha*, two morphologically similar murid rodent species that have been implicated in the transmission of bubonic plague. In order to unequivocally identify South African rodent species, complete cytochrome-B gene sequences were generated for 13 rodent species captured primarily in Limpopo Province. Alignment of these sequences with others deposited in the Genbank database enabled the design of species-specific primers that permit rapid and cost-effective species identification by PCR. In addition, phylogenetic analysis revealed insights of ecological, taxonomical and epidemiological significance such as an overlap in the distribution of *M. coucha* and *M. natalensis* in Limpopo Province, the paraphyletic status of the genus *Aethomys* and the presence of three commensal rat species in South Africa, one of which, the Asian *Rattus tanezumi* represents the first record of this species in Africa. These results are of importance for pest control, particularly in rural communities of developing countries, where rodents not only pose a zoonotic threat, but where they also compete with humans for food.

INTRODUCTION

By causing damage to crops, stored food, spreading diseases, and in destroying man-made infrastructures, rodents, particularly those of the family Muridae have been a menace to humankind for centuries worldwide (Leirs 1995). Consequently, an array of strategies to curb the devastating impact of murid rodents have been implemented over centuries. These range from the use of simple trapping devices, natural predators and common poisons to sophisticated anticoagulants which have led to the development of rodent control industry sectors in most countries (Leirs 1995).

Nevetheless, rodent pest problems still remain and are particularly prevalent in the developing world. It is only recently that researchers are re-evaluating rodent management approaches by developing ecologically-based management strategies (Singleton et al. 1999). As part of broader European Commission and DFID-funded research projects on rodents in southern Africa, the present study focuses on a community participatory research programme in four villages in Limpopo Province, South Africa. The objectives of the research project are to understand the ecology of rodents affecting people's livelihoods and to evaluate potential control strategies for their cost-benefits.

Approximately 50 murid rodent species are known to occur in South Africa (Skinner & Chimimba in press). Some of these, such as members of the genera *Rattus*, *Mastomys*, and *Tatera*, have been implicated in disease transmission and in causing damage to crops and stored grain

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(Venturi et al. 2004). This problem has been exacerbated further by the discovery of cytogenetically diverse and yet morphologically indistinguishable species over the last 25 years (Skinner & Chimimba in press). Consequently, complete cytochrome-B gene sequences were used in an attempt to cost-effectively and positively identify South African cryptic murid rodent species that may assist health and agricultural authorities in the identification of potentially problematic cryptic rodents in South Africa.

MATERIALS AND METHODS

Sampling strategy and area

Murid rodents were trapped in four villages Bloublommetjieskloof, Mapate, Nkomo-B and GaPhaahla in Limpopo Province, South Africa. Ten snap traps were placed in ten community households of each village and inspected daily.

Genetic characterisation

Total genomic DNA was extracted from liver or kidney samples of murid rodents using the High Pure PCR template preparation kit (Roche). The entire cytochrome-B gene was amplified using murid rodent primers that bind within flanking gene regions (Ducroz et al. 1998). The resulting amplicon, approximately 1.2 kb in size was purified directly from the tube using the High Pure PCR product purification kit (Roche) according to manufacturer specifications. Nucleotide sequences were determined with both PCR primers, at an annealing temperature of 48°C using version 3.0 of the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer).

Species-specific primer design

Complete cytochrome-B gene sequences were generated for at least two representatives of each of the 11 species captured. Of particular importance from a species-identification viewpoint was the need to distinguish between the two medically important, but cryptic species of the genus *Mastomys* (Venturi et al. 2004), the sibling species of the genus *Aethomys* (Linzey et al. 2003), and all representatives of the genus *Rattus* that occur in South Africa. As striped mice (*Lemniscomys rosalia* and *Rhabdomys pumilio*) are easily distinguishable by the number of their stripes and pelage colour patterns, there was no need to design species-specific primers. However, because the two gerbil species (*Tatera leucogaster* and *Tatera brantsii*) are sometimes mistaken for *Aethomys* in the field due to the similarity in pelage colour, there was a need to design species-specific primers capable of differentiating between individuals from these two genera.

All nucleotide sequences from this study were aligned using the DAPSA programme (Harley 2001), and this final dataset was complemented with 14 additional full-length sequences of African murid rodent species, that have been deposited in the Genbank database. A universal primer capable of binding to and amplifying all 15 species was identified. Subsequent to this, species-specific primers permitting within-genus resolution, of *Aethomys*, *Tatera*, *Rattus* and *Mastomys*, were selected in such a manner that each species was identifiable by a unique PCR product size, whilst ensuring that primer annealing temperatures were sufficiently similar to allow for multiplexing of reactions.

Phylogenetic analysis

Complete gene sequences of South African murid rodents identified in this and previous studies were used to infer a phylogeny. Summary statistics for the nucleotide and amino acid data were obtained in MEGA2 (Kumar et al. 2001). Hierarchical likelihood ratio tests were performed with Model Test 3.5 in order to identify which of the 56 possible models of sequence evolution best fitted the nucleotide data at hand (Posada & Crandall, 1998). The model and data parameters identified under the Akaike Information Criterion (AIC) were subsequently used for Minimum Evolution (ME) and Maximum Likelihood (ML) analyses. An amino acid (aa) dataset 380 aa in

length was also used to infer a phylogeny. Nodal support for clades was assessed by bootstrap resampling.

Morphological assessment

All positively-identified specimens were also examined to assess qualitative morphological differences between them. These included the examination of cranial, mandibular, and dental traits, and with reference to existing identification keys (Skinner & Chimimba in press).

RESULTS

Of the 1140 sites in the nucleotide dataset, 510 (44.7 %) were not completely conserved across all taxa. Of these variable sites, 467 were parsimony informative and 43 were singletons. For the deduced amino acid dataset, 110 (28.9 %) of the total of 380 sites varied, of which 95 were parsimony informative and 15 were singletons. The average nucleotide composition was 29.6 %, 26.8 %, 31.2 % and 12.4 % for T, C, A and G, respectively, revealing a strong AT-bias (60.8 %).

Eight genera comprising eleven murid rodent species were identified by phylogenetic analysis of the amino acids sequences of the mitochondrial cytochrome-B gene (Fig.1). These included *Mastomys natalensis* and *M. coucha*, *Rhabdomys pumilio*, *Lemniscomys rosalia*, *Aethomys namaquensis, A. ineptus*, members of the genera *Tatera, Steatomys* and *Saccostomus*, as well as *Rattus rattus* and the Asian *R. tanezumi*. Support for species clades (on terminal branches) was generally good (between 88 % and 100 %), however the internal branches generally had very low bootstrap support, making it impossible to discern intra-generic relationships particularly within the sub-family Murinae. The identification of *Rattus tanezumi* in this study represents the first record of this species in Africa, and increases the total number of commensal rat species to three, viz. *R. rattus*, *R. norvegicus* and *R. tanezumi*. The phylogenetic results further revealed that the species currently considered congenerics of the genus *Aethomys* are genetically distinct and fall within two separate, unrelated clades - one consisting of *A. namequensis* (99 % boostrap support), and the other comprising *A. ineptus* and *A. chrysophilus* (98 % boostrap support). The latter clade was of further interest as *A. ineptus* and *A. chrysophilus* although morphologically indistinguishable were readily discerned from nucleotide sequences (results not shown). However, no differences were noted between the species on amino acid level (Fig. 1).

While most of the species could be identified morphologically using existing identification keys, *M. natalensis* and *M. coucha* were indistinguishable. Examination of the skulls of the 14 positively-identified *Rattus* specimens did however reveal what appears to be consistent differences between the incisive foramina of *R. rattus* and *R. tanezumi*.

Based on the results obtained from sequencing in excess of 100 murid rodent species, the need to design three multiplex PCRs was identified. Plex I consisted of three primer sets capable of discerning individuals from three discrete genetic groups, namely those from (i) *Tatera*, (ii) *A. namaquensis* and (iii) the *A. chrysophilus-A. ineptus* species complex. Plexes II and III comprised of two sets of primers each, with the former distinguishing between *M. coucha* from *M. natalensis* and the latter between *R. rattus* and *R. tanezumi*. Each of the plexes resulted in PCR products of distinctive sizes, making it possible to readily distinguish between species (results not shown).

DISCUSSION

The phylogenetic results revealed the presence of 10 murid rodent species within the sampling sites in Limpopo Province. Of these, the need to distinguish between the two *Mastomys* species, between the two *Aethomys* species and *Tatera* (sometimes misidentified as an *Aethomys*), as well as between the two *Rattus* species, one of which was previously not known to occur in South Africa, was identified. Three multiplex reactions were subsequently developed which allow for species identification in a cost- and time-effective manner. The benefit of the PCR approach detailed in this study is that larger sample sizes can be screened, which will not only assist in the identification of

the murid rodent species that pose the greatest zoonotic threat in South Africa, but will also result in more accurate species distribution maps. This latter advantage has already resulted in the need to revise *Mastomys coucha* and *M. natalensis* distribution maps, as these two species were previously not believed to co-occur in this region.

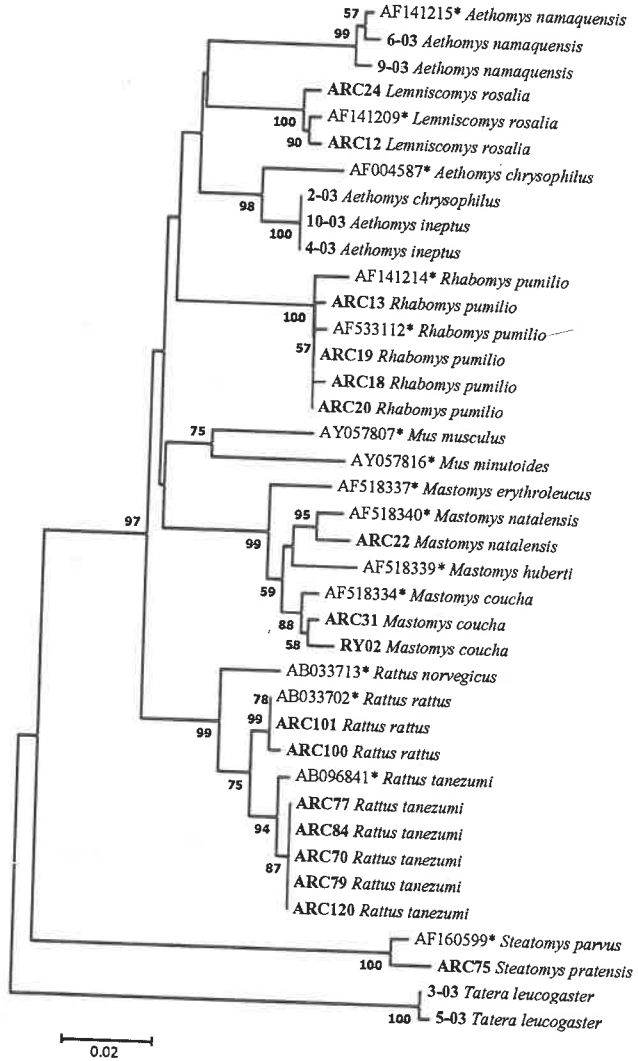

Fig. 1. Cytochrome-B gene tree based on complete amino acid sequences of African murid rodent sequences generated in this study (indicated in bold), and those obtained from the Genbank database (denoted with a *). Boostrap values >50 are indicated next to the relevant node

The Asian *Rattus tanezumi* was identified for the first time in South Africa, in particular and Africa in general and was shown to co-occur with *R. rattus*. The availability of genetically positively-identified specimens, permitted a morphological comparison. Preliminary results indicate that *R. rattus* and *R. tanezumi* appear to differ in the posterior incisive foramina region. However, this difference needs to be treated with caution as it only involved a few positively identified specimens and may therefore not reflect the extent of morphological variation in these species. It is proposed that a further traditional and geometric morphometric investigation with additional samples be undertaken in an attempt to assess the nature and extent of morphological variation in both *R. rattus* and *R. tanezumi*.

Apart from the positive identification of murid rodents in this study, an interesting result was the placement of currently considered con-generics of the genus *Aethomys* in separate clades, suggesting that the genus is paraphyletic. These results are more consistent with the traditional subdivision of the genus *Aethomys* into two subgenera, namely the subgenus *Micaelamys* and the nominate subgenus *Aethomys*, with the former subgenus only comprising *A. namaquensis* and *A. granti* (Ellerman et al. 1953). Together with microcomplement fixation of albumin data (Watts & Baverstock 1995) that showed *A. namaquensis* to be immunologically distant from *A. chrysophilus* and more recent mitochondrial DNA and 16S rRNA data (Ducroz et al. 2001; Castiglia et al. 2003) that placed *A. namaquensis* and *A. chrysophilus* in separate clades, taxonomic revision of this genus may be warranted. As all these results suggest that the genus *Aethomys* is paraphyletic, elevation of the currently recognized subgenera *Micaelamys* and *Aethomys* to full generic rank is suggested, particularly as *Micaelamys* and *Aethomys* have been shown to be distinct in other studies on cranial and dental morphology, modes of karyotypic change, and gross sperm and bacular morphology (Ellerman et al. 1953; Matthey 1964; Visser & Robinson 1986, 1987; Chimimba in press). Apart from differences in general body and cranial size, other differences include a more robust skull form with members of the genus *Aethomys* having more prominent cranial sutures, while members of the genus *Micaelamys* have a well-developed anterior median cusp on the first upper molar (Chimimba in press).

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PIONEERING MANAGEMENT OF THE ADULT BLACKFLY, *SIMULIUM CHUTTERI* (DIPTERA: SIMULIIDAE): A THREAT OF MEDICAL AND VETERINARY SIGNIFICANCE IN SOUTH AFRICA

V.L. Hobololo[1], K. Kappmeier-Green[1] & B.L. Penzhorn[2]

ABSTRACT

The historical focus on blackfly control in South Africa has exclusively been on the aquatic phase of the life cycle. Despite tremendous efforts to apply these techniques, the shortcomings of their application are evident in that blackfly are still a problem along the major rivers in South Africa. The most recent outbreaks occurred in 2004/5 in the Bloemhof/Hoopstad districts. Farming communities situated in the surroundings of the North West/Free State Province border were severely affected. Following the risk-assessment study in various districts along the Vaal River, research was initiated at controlling the adult blackfly in surrounding the farming communities. Candidate pesticides were evaluated for efficacy against blackflies in feedlots in the sheep-farming communities. Preliminary results indicated the value of these pesticides and of the feedlots to protect sheep from blackfly attacks. The risk assessment survey conducted along the Vaal River also indicated that gross economic losses were experienced by the tourism industry. In several cases tourists were hospitalized due to allergic reactions to blackfly bites, which affected the industry.

INTRODUCTION

Adult female blackflies require a blood meal (Welton *et al.*, 1987; Palmer, 1997; Gibson & Torr, 1999) for ovarian development (Davies & Peterson, 1956; Peterson, 1994; Crosskey, 1990). Due to their blood-feeding activity, they are considered ideal disease transmitters (Crosskey, 1990) and are best known for transmitting the filarial nematode worm *Onchocerca volvulus* to humans (Nelson, 1991; Davies, 1994; Hougard *et al.*, 1997; Gibson & Torr, 1999). The resulting disease known as onchocerciasis or river blindness has left more than 20 million people infected and millions more blind in West Africa and South America (Rodriquez-Perez *et al.*, 1995; Samba, 1995; Hougard *et al.*, 1997; Molyneux & Davies, 1997) although it has not been reported in South Africa yet. Furthermore, in humans, the bites of some blackfly species can cause allergic reactions known as blackfly fever or simuliotoxis (Crosskey, 1990; Palmer, 1997). This is characterized by swelling, itching, haemorrhage and oedema (De Villiers, 1987), which require medical attention in severe cases (Mason & Shemanschuk, 1990).

Since 1950 frequent blackfly outbreaks have been reported from the Vaal River (Howell & Holmes, 1969; Nevill, 1988) and subsequently *S. chutteri* (Chutter, 1968; Howell & Holmes, 1969), *S. damnosum s.l.*, *S. nigritarse* (Steenkamp, 1972) and *S. adersi* (Begemann, 1980) were identified as pest species. In livestock, blackflies readily attack the exposed parts of the body, e.g the eyes, ears and udders (Anderson & Voskuil, 1963) and resulting wounds are prone to secondary infections, which sometimes lead to the death of animals (Palmer, 1997). In South Africa, simuliids have been implicated in the spread of two pathogens to animals. These are *Chlamydia* sp., that cause blindness in sheep and abortion in cattle (de Moor, 1982), and the Rift Valley Fever virus, which led to major Rift Valley Fever outbreaks between Prieska and Groblershoop in 1975 (McIntosh *et al.*, 1980).

In addition, blackflies cause considerable irritation to livestock (Anderson & Voskuil, 1963; Crosskey, 1990; Kok *et al.*, 1994). Sheep under blackfly attack will huddle together with their head

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stuck underneath each other. During these periods, no feeding or mating occurs, thus resulting in decreased weight gain indices and reduced lambing percentages (Palmer, 1997). Blackfly annoyance furthermore leads to economic losses through reduced efficiency of agricultural and industrial workers, interference in recreation, and reduced real estate values (Mason & Schemanschunk, 1990).

Although there are numerous reports of blackfly epidemics in South Africa, only Steenkamp (1972) has made a detailed study on the economic importance of the pest. Blackfly control methods used in South Africa to date have exclusively focused on intercepting the aquatic immature stage of the life-cycle (Chutter, 1968; O'Keffe, 1985; de Moor & Car, 1986). This involved aerial spraying of the river systems with larvicides Howell & Holmes, 1969; Undeen *et al.*, 1981; Lacey *et al.*, 1982; de Moor, 1986), regulation of river flow rates (de Moor, 1982; de Moor & Car, 1986) and water-level manipulation (Howell *et al.*, 1981; Car, 1983) in order to prevent larval breeding and development.

New control methods that aim at targeting the adult stage are being studied as a contribution towards an area-wide integrated pest management of blackfly. Different insecticides have therefore been suggested by several agrochemical manufacturers to evaluate their value in protection of livestock from blackfly attacks. The purpose of this study was therefore to test the efficacy of some pesticides to this pest, discuss economic implications that relate to medical impact of the insect pest in humans and recommend further potential solutions to both veterinary and medical problems.

METHODS

Pesticide Efficacy

Following a survey conducted along the Vaal River (Hobololo *et al.*, 2005) in 2004/5, it was indicated that the Warrenton – Christiana – Bloemhof area is a high risk zone for blackfly infestation (Fig.1). Consequently, three candidate pesticides were tested in feedlots at Springfield farm (E 24°52′ S 28°19′), situated at about 4km from the Vaal River near Warrenton.

These products were Delete®, Delete All® and Ektopor®, with the following active ingredients: Delete® : 0.5% deltamethrin, 2% piperonyl butoxide (PBO); Delete All® : 0.5% deltamethrin, 2% PBO and 2% amitraz; and Ektopor®: high-cis cypermethrin 20g/l. The weight-related (Delete®: 1ml/10kg; Delete All®: 1ml/10kg; Ektopor®: 10ml up to 100kg) dosages of these pesticides were calculated for individual animals and applied accordingly in target body parts as a standard method required by the manufacturers.

Sixty sheep (black-head Dorpers and white wool Merinos) were randomly divided into 4 groups of 15, three of the groups being treatments and the fourth being the control. Four parameters were recorded prior and during the trial, these included : total body mass of each animal in the group, ear scab profiles, annoyance level indicators (ALIs) and blackfly abundance.

Body weight

Body weight was monitored in all experimental and control animals on a weekly basis for a period of 3 weeks. This is because weight loss is a typical phenomenon in blackfly-infested animals, since the pest's annoyance, amongst other things, disturb the feeding behaviour of the animals. Each animal was weighed by suspending its total mass on a SALTER suspended weigher, Model 235 (150 kg x 500 g) scale. The animals were maintained on a standard diet from Lubern Stock Feeds (Pty) Ltd., with the following nutritional content : Protein – 120g/kg (min.), Ammonium Chloride (derivative) 16.4% (max.), Ammonium Chloride 12g/kg (max.), Fat Content 25g/kg (min.), Fibre 200 g/kg (max.), Calcium 10g/kg (max.), Phosphorus 2 g/kg (min.) and Oxygen 120 g/kg (max.). Water supply was adequately maintained throughout the course of the study. Data were analysed using the Dunnett Multiple Comparison test of the GraphPad Instat© software.

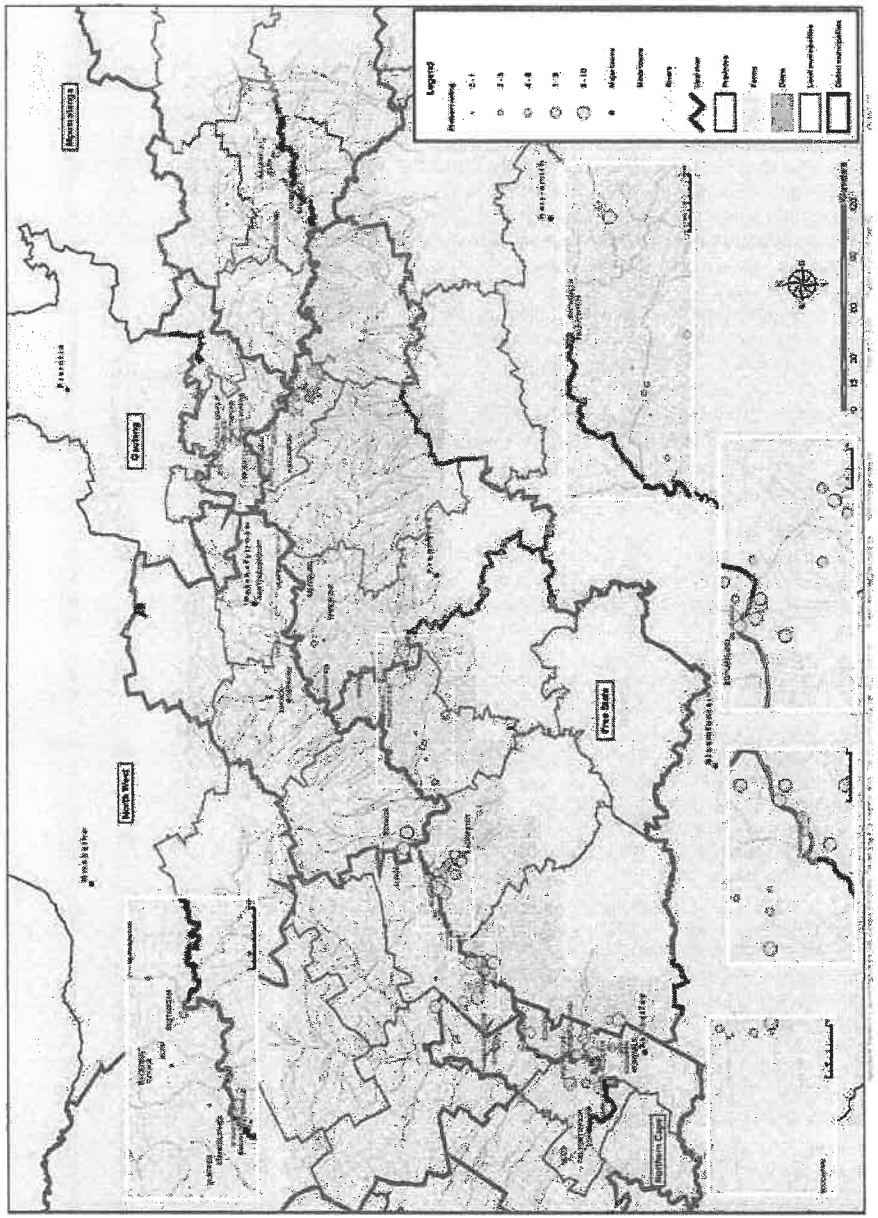

Fig. 1. Indication of high risk zones for blackfly attacks along the Vaal River, South Africa

Ear Scab Profiles

The inner ears of animals are preferred biting sites for *Simulium* spp. (Palmer, 1997) and therefore sheep were categorised on a 6-point scale in relation to the extent of damage blackflies had caused to their ears in the form of scabs. This ranged from :
0 = no lesions visible, 1 = minimal lesions, 2 = obvious but less than 20% of the ear with crusts and inflammation, 3 = conspicuous lesions <50%, 4 = extensive lesions <75%, to
5 = entire inside ear with lesions, >75% crusts, ears swollen and may lead to secondary infection. The ear scab profiles were monitored weekly for a period of 3 weeks.

Annoyance Level Indicators (ALIs)

Ear twitches, foot stomping and associated behaviours are reactive behavioural patterns of sheep to blackfly annoyance (Palmer, 1997). These were measured per 3-minute cycles using a stop watch and Bushnell 12 x 50 binoculars. The ALIs were recorded on a daily basis. Data were analysed using Tukey-Kramer Multiple Comparison test of the GraphPad Instat© software.

Blackfly Abundance

Blackflies can disperse up to more than 80 kilometers from the river bank (Palmer, 1997), and the Springfield feedlots used in this study were only about 4 km from the Vaal bank. A standard number (20) of sweeps were made in each feedlot (group) daily using hand-nets to monitor abundance of blackfly populations pre- and post treatment.

Economic impact on the tourism industry

Protea Hotel self-catering resort, Nkolo Spa is the most popular stop-over and conference centre between Cape Town, Kimberely and Gauteng on the N12 offering rooms, self-catering chalets and caravan parks for camping. The resort is set in lush, secluded surroundings on the banks of the Vaal River, situated in a game reserve which stretches over 23 000 hectares and is well-stocked with game including white rhino, zebra and a selection of antelopes.

Although no disease transmission was reported, a large number of guests experienced allergic reactions, were hospitalized and had to cancel their vacations due to blackfly attacks on humans. Economic losses suffered by this resort from January to May 2005 is given.

RESULTS AND DISCUSSION

Pesticide Efficacy

Body weight

Insecticide application has proven to possess some value in blackfly control for livestock, especially when used not in isolation but within an integrated pest management context. Of the products tested for efficacy in sheep, Delete® has produced better results (Table 1 & 2). The Dunnett Multiple Comparison Test ($p < 0.05$) indicated that only Delete® treatment had significant difference in weight gain when compared against the control with two other treatments (Delete All® and Ektopor®). The average weekly measured weight gain indices were also significantly higher for Delete®. No significant difference was evident in comparison of the each of the other 2 treatments against the control (Table 1).

Table 1. Average body mass (kg) of the sheep pre- and post treatment

	Delete All®	Ektopor®	Delete®	Control
Pre-treatment	30.6	27.5	30.1	29.6
Post-treatment(Week 1)	34.6	28.6	33.4	30.3
Post-treatment (Week 2)	32.1	30.4	34.1	31.6
Post -treatment (Week 3)	34.7	31.6	35.1	33.7

Table 2. Body weight gain analysis for all four groups of sheep in separate feedlots (3 treatments and control)

Comparison	Mean Difference	q	p - value
Control vs Delete All	-1.7	2. 275	$p > 0.05$
Control vs Ektopor	1.667	2.23	$p > 0.05$
Control vs Delete	-2.33	3.122	$p < 0.05$

If the value of q is greater than 3.100, then p-value is less than 0.05.
For repeated Means of ANOVA, p – value = 0.0066, considered very significant.

Ear Scab Profiles

Ear scab profiles from week 1 to 3 (Fig. 2a – c) also succeeded in confirming better efficacy for Delete® in that initially there were no scabs at all in this group for 2 weeks post treatment. It is only in third week that one sheep was affected, even then only minimally. However, for other groups there was a slight increase in ear scabs after 1 to 3 weeks but only limited to category 1 (minimal lesions). Four sheep from the control group also also showed scab categories 1 to 4. The occurrence of the minimal scabs in the one sheep from the Delete®-treated group could have residual effect interval implications.

ALIs

The Tukey-Kramer Multiple Comparison Test indicated no significant difference ($p > 0.05$) for the annoyance level indicators even though the average ALI per 3-min cycles were lower for the Delete® treatment group (Table 3). The annoyance level was reasonably high for all four groups (Table 4). The reason for this could be that, in the feedlots there is usually presence of other insect pests, e.g houseflies, *Musca domestica* and these are also capable of causing ear twitching and annoyance to sheep although it might not result in damages similar to those caused by the blackfly. Therefore, use of ear scab profile analysis may be more accurate than ALI per 3-min cycle measurement since the former measures the actual damage caused by blackflies and therefore rules out the effect of annoyance by other insects except blackfly.

Another interesting field observation was that even though the animals in all four groups were a mixture of black-head Dorpers and white wool Merinos, scabs were almost completely biased towards the black-head Dorpers. The same phenomenon was observed when counting ALIs per 3-minute cycles, that many ear-twitches were recorded from black-head Dorpers than on Merinos. This might suggest that landing preferences for the annoying species could be colour driven.

Table 3. Annoyance level indicator analysis for at the average frequency of 3-min per cycle

Comparison	Mean Difference	q	p-value
Delete All vs Ectopor	7.158	0.882	$p > 0.05$
Delete All vs Delete	22.142	2.729	$p > 0.05$
Delete All vs Control	15.292	1.885	$p > 0.05$
Ectopor vs Delete	14.983	1.847	$p > 0.05$
Ectopor vs Control	8.133	1.003	$p > 0.05$
Delete vs Control	-6.85	0.844	$p > 0.05$

One-Way ANOVA : p-value is 0.267, considered not significant.

Table 4. Average annoyance level indicators per 3-minute cycles

	Delete All®	Ektopor®	Delete®	Control
	29.2	21.2	11.7	17.1
	13.3	17.7	23.3	12
	50.5	73	13	30.4
	81	53.7	46.9	51.7
	67.3	39.4	27.2	41
	27.1	20.4	13.4	24.4
Means	**44.7**	**37.6**	**22.6**	**29.4**

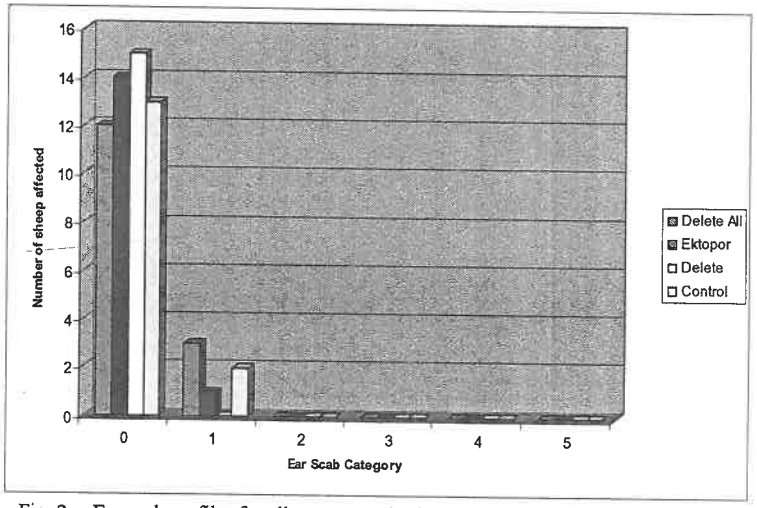

Fig. 2a. Ear scab profiles for all groups on the first week. (Week 1)

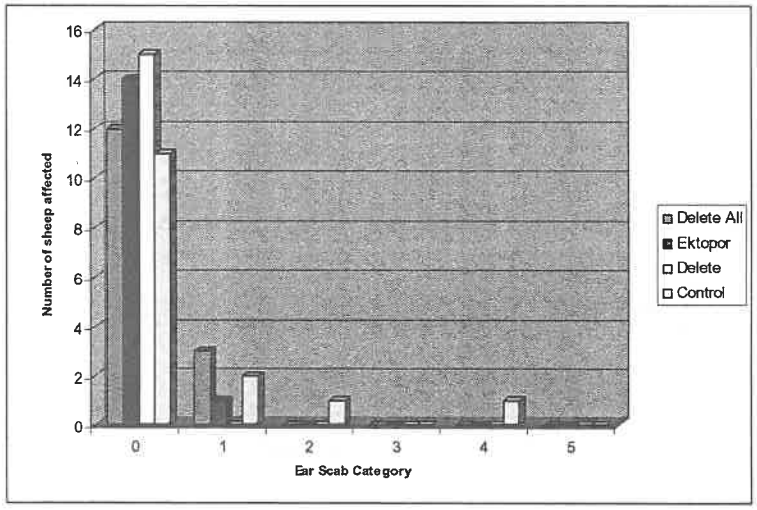

Fig. 2b. Ear scab profiles for all groups on the second week (Week 2)

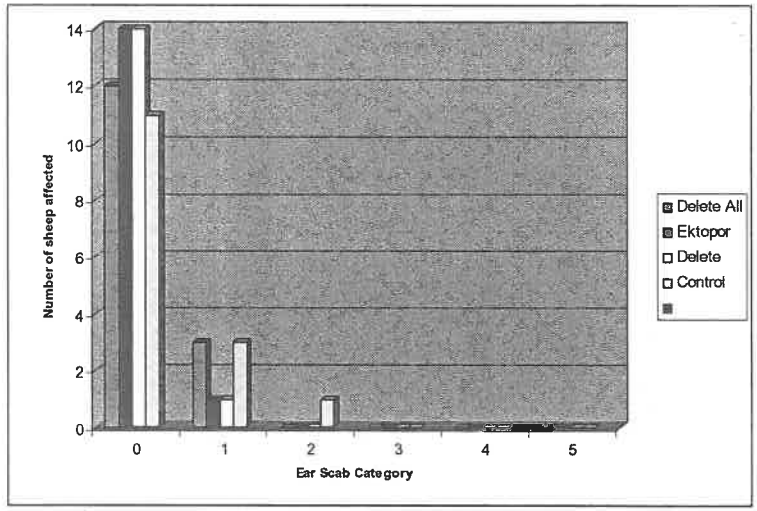

Fig. 2c. Ear scab profiles for all groups on the third week (Week 3)

Blackfly Abundance

In terms of blackfly abundance (Fig. 3), the use of handnets is probably not a very reliable method for monitoring pest populations. A greater challenge is development of an effective trapping technology which can be used for monitoring and probably also tested for control value.

Future endeavours towards controlling the adult blackfly annoyance in sheep should focus in continuing efficacy studies in open fields and not in feedlots. Feedlot farming should also be recommended for farming communities which can afford it as it seems to decrease the level of vulnerability of livestock to blackfly. Further studies on baiting systems derived from chemical ecology bio-assays and integration of the aquatic immature stage lifecycle control techniques with adult stage interventions might also bring fruitful results.

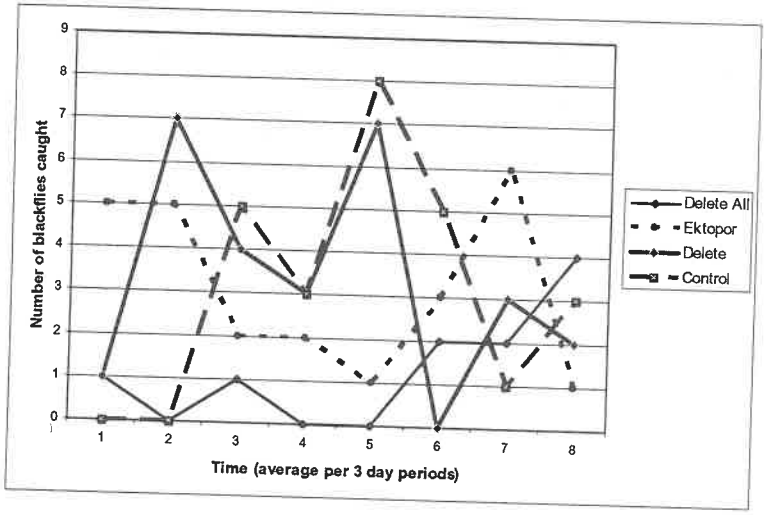

Fig. 3. Average (per 3-day interval) blackfly catches using handnets

Economic impact on the tourism industry

The medical implications of blackfly attacks in humans continue to grossly affect the tourism industry. During the risk assessment survey (Hobololo *et al*, 2005) conducted along the Vaal many holiday destinations indicated that the guests were forced to end their vacation since many of them had to be hospitalized from allergic reactions due to blackfly bites. Some resorts had to close during the time of the outbreak and only to re-open halfway through the year when pest populations were relatively lower. Nkolo Spa being the most popular resort with highest capacity was used as a model in this study. Compared to the chalets and hotel rooms, the caravan park guests experienced most problems and were often forced to leave before the booked intervals. The total loss suffered in refunds due to blackfly attacks only from January to May 2005 amounted to R 35 200.00 (Fig. 4).

Furthermore, the capacity of this caravan park per month is 40 800 guests or 1360 per day. However, the average bookings in the caravan park only averaged at 950 per month for the January – May 2005 period. Under ideal conditions where the caravan park would be fully booked for the whole month, Nkolo Spa would be making almost R8-million rands (derived from the 40 800 monthly guest capacity at R200 rate/day) more than the stated current average.

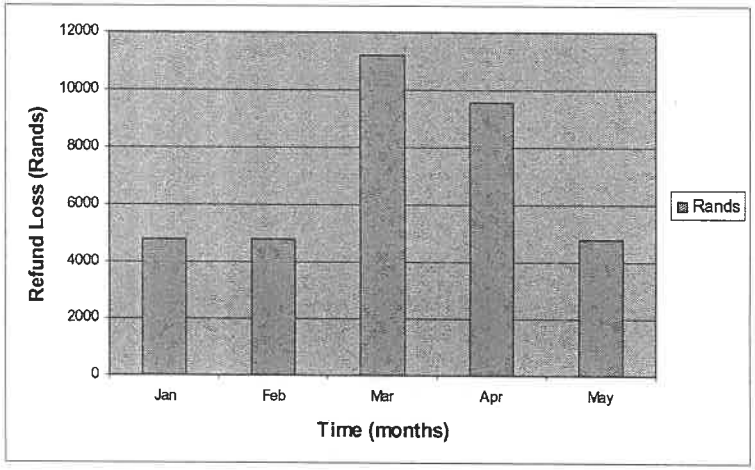

Fig. 4. Nkolo Spa refund losses in 2005 due to blackfly attacks on humans (caravan park)

It is recommended that the National Department of Health be involved in blackfly control through institutions such as the Medical Research Council (MRC) since this institution could have better expertise of addressing the medical impact of this pest in humans. However, the Agricultural Research Council – Onderstepoort Veterinary Institute will continue in developing methods for controlling this pest at adult stage and addressing other veterinary implications.

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GENETIC PROFILES OF *MYCOBACTERIUM BOVIS* IN CATTLE IN SOUTH AFRICA

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ABSTRACT

Genotyping based on IS*6110* and PGRS RFLP as well as spoligotyping was performed on 57 *M. bovis* isolates from reactor cattle in 37 farms in South Africa and one isolate from Swaziland. PGRS RFLP proved to be the most discriminatory method, followed by IS*6110*. The presence of two genetically unrelated strains was demonstrated for five out of eleven farms for which more than one isolate was available for analysis.

INTRODUCTION

8 of 9 provinces now +ve herds

Bovine tuberculosis is believed to be introduced to South Africa and possibly the sub-region by European settlers (Hutcheon 1880). At the beginning of the 20th century cattle imports from Australia, Argentine and Madagascar were often found to contain infected animals (Cousins et al. 2004). The introduction of a National Tuberculosis Scheme in 1969 resulted in reduction of the BTB prevalence to below 0.01% in commercial cattle herds within 2 decades but new outbreaks continued to occur (Michel 2002). Between 1996 and 2003 the number of reported infected cattle herds increased from 6 to 20 (Official statistics of the National Department of Agriculture) . Presently the control of bovine tuberculosis is partially based on intradermal tuberculin testing and partially on abattoir surveillance. In the latter case detection of suspect lesions is followed by trace back of herds which are subjected to the test-and-slaughter programme. Chronic cases.

Traditional epidemiological investigation of disease outbreaks is often hampered by the lack of tools to trace back the source of infection. The introduction of molecular typing techniques has provided powerful tools to study transmission of disease agents as well as the efficacy of control measures (van Embden et al. 1993). Despite a lack of consensus on the 'best' technique, genetic typing of *M. bovis* has repeatedly been proven useful in studying the epidemiology of human tuberculosis from tracing back outbreaks to an increased understanding of the global distribution of *M. tuberculosis* strains (Glynn et al. 2002, Lockman et al. 2001). DNA fingerprinting techniques have also helped to elucidate interbovine and interspecies transmission of *M. bovis*. Such information is crucial to bovine tuberculosis control schemes and the effective management of the wildlife-livestock interface in countries where wildlife reservoirs for *M. bovis* have been identified (Haddad 2004, Durr 2000, Skuce & Neill 2001, Serraino 1999, Costello 1999). The most widely used techniques include IS*6110* Restriction Fragment Length Polymorphism (RFLP) typing, the polymorphic 'GC-rich repeat' sequence (PGRS) typing, spoligotyping and VNTR (variable number of tandem repeat) typing. In the present study we used IS*6110* RFLP, PGRS RFLP typing as well as spoligotyping to describe the genetic profiles of the pathogen involved, *M. bovis* as isolated from local, domestic cattle between 1993 and 2000.

Buffalo were originally infected when they broke out and mingled with a positive herd. That farm is now depopulated.

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MATERIALS AND METHODS

Bacterial isolates

Fifty-seven *Mycobacterium bovis* isolates originating from 37 different cattle herds in six provinces of South Africa were used in this study (see Table 1). An additional strain (631) was isolated from a bovine with suspect lesions in Swaziland. All diagnostic samples were processed for culture according to standard procedures (Bengis et al. 1996). Mycobacterial isolates were identified as *M. bovis* using polymerase chain reaction (PCR) and biochemical reactions (Alexander et al. 2002). In all cases where DNA fingerprinting could not be carried out directly after isolation, pure subcultures of *M. bovis* on Loewenstein-Jensen medium containing pyruvate were stored at -20°C.

Figure 1 indicates the farms of origin of the 56 *M. bovis* isolates.

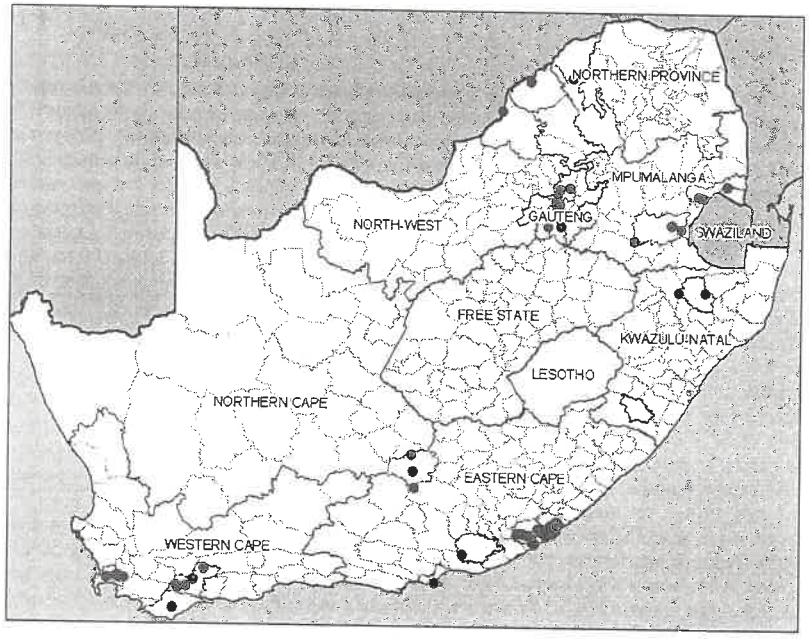

Figure 1: Distribution of *Mycobacterium bovis* isolates analysed by gentoyping

DNA extraction

DNA extraction of *M. bovis* isolates was accomplished from colonies subcultured onto Lowenstein Jensen medium. Following heat-inactivation at 80 °C for 60 minutes, the colonies were scraped off and suspended in 5 ml of extraction buffer (50g/l Mono Sodium Glutamic Acid; 6.06g/l Tris.HCl (pH 7.4); 9.3g/l EDTA); (Warren, pers. comm..) to which Lysozyme ([50mg/ml]) and RNAseA ([10mg/ml]) were added. The suspension was incubated for 2 hours at 37 °C after which Proteinase K at a final concentration of 0.5mg/ml was added. The suspension was incubated at 45 °C overnight followed by phenol/chloroform extraction. The final pellet was resuspended in 40µl TE (1mM Tris.HCl (pH 7.6); 0.1 mM EDTA).

IS*6110* typing

For IS*6110* typing approximately 1.5 µg of mycobacterial DNA was digested overnight with 1.5 units of *Pvu* II,subsequently the resulting fragments were separated by electrophoreses on a 0.8% agarose gel. DIG-labeled molecular weight size marker VII (Roche) was loaded in the first, middle and last lane of the gel. Southern blot transfer was performed as described by Skuce et al. 1994. Hybridisation and detection of IS*6110* containing DNA fragments was performed according to the manufacturer's instructions (*Roche Molecular Biochemicals-The DIG System User's Guide*,1995). The GelComparII software (Applied Maths, Kortrjik, Belgium) was used to compare hybridization patterns obtained by IS*6110* RFLP typing and spoligotyping (similarity coefficient of Dice, unweighted pair group method with arithmetic averages [UPGMA], UPGMA, maximum tolerance 1.2%).

PGRS RFLP typing

For PGRS RFLP typing 1.5µg of *M. bovis* DNA was digested with Alu I and electrophoresis of DNA fragments was performed on a 1.2% agarose gel. Southern blotting and detection were performed as described for IS*6110*. Hybridisation with DIG-labelled PGRS oligonucleotide probe was performed at 55°C.

Spoligotyping was kindly performed by Kristin Kremer (National institute of Public Health and the Environment, Bilthoven, the Netherlands).

RESULTS

Genotyping of *M. bovis* isolates

Complete data sets containing information regarding IS*6110* and PGRS RFLP as well as spoligotyping were obtained for six *M. bovis* isolates relating to five outbreaks. Due to technical limitations other isolates could be subjected only to one or two of the three typing methods. The results are summarized in Table 1 and Table 2.

IS*6110* RFLP

Among 50 *M. bovis* isolates from 34 farms probed with IS*6110*, 17 different banding patterns were identified (Fig. 2). They comprised four to ten bands, which, due to the fact that the entire sequence of IS*6110* was used as a probe as described previously (Skuce 1994), relates to two to five copies of IS*6110*. Each of eleven RFLP patterns was associated with a different BTB outbreak (IS*6110* types C4, C5, C6, C7, C9, C10, C11, C13, C15, C16, C17), three patterns were each found to be involved in 2 unrelated outbreaks (C3, C8, C14) and two patterns, both resembling *M. bovis* strains with two copies of IS*6110*, were found in six and eleven unrelated outbreaks, respectively (C1 & C2). One genotype characterised by six bands was isolated from two related outbreaks (C12).

Table 1. DNA fingerprinting results for 58 *M. bovis* isolates

Sample ID	IS*6110*	Spoligotype	PGRS	Province	Farm	Year of isolation
1255	C1	ND	J	EC	1	1998
1130	C1	ND	ND	EC	2	1997
1729	C2	ND	C	EC	3	2000
1730	C2	ND	C	EC	3	2000
1967	C2	ND	ND	EC	5	2000
1048	C6	ND	ND	EC	6	1997
1057	C16	ND	ND	GP	11	1997
1078	C16	ND	I	GP	11	1997
1202	C16	ND	I	GP	11	1998
1311	C16	ND	I	GP	11	1998

Sample ID	IS*6110*	Spoligotype	PGRS	Province	Farm	Year of isolation
1307	ND	ND	R	GP	11	1998
1497	C2	ND	H	GP	12	1999
1502	C7	ND	P	GP	13	1999
1541	C9	ND	ND	KZN	16	1999
1760	C1	ND	G	LP	19	2000
1762	ND	ND	G	LP	19	2000
1763	C1	ND	G	LP	19	2000
781	C1	ND	ND	LP	20	1996
709	C4	ND	ND	LP	21	1996
392/94	C1	ND	S	MP	24	1994
187	C1	ND	ND	EC	25	1994
1226	C17	ND	Q	MP	26	1998
1228	C12	ND	M	MP	26	1998
1302	C12	ND	M	MP	27	1998
1374	ND	ND	M	MP	27	1998
1458	C14	ND	ND	MP	28	1999
681	C2	ND	F	WC	31	1994
1450	C2	ND	ND	WC	32	1999
1341	C5	ND	L	WC	33	1998
1757	C8	ND	O	WC	34	2000
213B/94	C11	SP1	ND	LP	22	1994
213D	C11	SP1	ND	LP	22	1994
871H/93	C8	SP1	E	MP	29	1993
871J/93	C8	SP1	E	MP	29	1993
1268	ND	ND	D	WC	4	1998
463	C1	SP2	ND	EC	9	1995
494	C1	SP2	ND	EC	9	1995
239	C1	SP2	ND	WC	14	1994
626	C1	SP2	ND	LP	23	1995
1725	ND	ND	T	EC	3	2000
810	C15	SP3	B	WC	35	1996
532	ND	SP4	ND	GP	15	1995
560	C1	SP4	ND	KZN	17	1995
614	ND	SP4	ND	KZN	18	1995
251E	C10	SP5	ND	EC	38	1994
251G	C10	SP5	ND	EC	38	1994
933	C3	SP6	N	EC	10	1997
934	C3	SP6	ND	EC	10	1997
506	ND	SP7	ND	EC	9	1995
568	C2	SP8	ND	EC	7	1995
615	C2	SP8	ND	EC	7	1995
623	C2	SP8	ND	EC	7	1996
876	C3	SP8	A	EC	7	1997
903	C3	SP8	ND	EC	8	1997
904	C3	SP8	K	EC	8	1997
631	C1	SP8	ND	SW	30	1996
268	C13	SP9	ND	WC	36	1994
999	C14	SP9	ND	WC	37	1997
58	18	9	20		37	

Table 2 shows that two outbreaks were each caused by two *M. bovis* strains with different IS*6110* patterns (C12 & C17 in herd 26; C2 & C3 in herd 7).

Table 2: Genetic relationship between *M. bovis* isolates in infected cattle herds

Farm No.	No. of isolates	IS*6110* type	PGRS type	Spoligotype
3	3	C2, C2,ND	C, C, T	ND
7	4	C2, C2, C2, C3	ND, ND, ND, A	SP8
9	3	C1, C1, ND	ND	SP2, SP2, SP7
10	2	C3	N, ND	SP6
11	5	C16, C16, C16, C16, ND	I, I, ND, I, R	ND
19	3	C1, C1, ND	G	ND
22	2	C11	ND	SP1
26	2	C12, C17	M, Q	ND
27	2	C12, ND	M	ND
29	2	C8	E	SP1
38	2	C10	ND	SP5

PGRS RFLP typing

PGRS typing of twenty-eight *M. bovis* isolates originating from 18 farms yielded 20 unique banding patterns. The involvement of two different genotypes in the same outbreak was confirmed for three herds. Genotypes M and Q were found in herd 26, C and T in herd 3 and I and R in herd 11. (see Table 1). One of these types, type M, was associated with two outbreaks (farms 26 and 27) in different provinces. A link between the outbreaks through movement of cattle between the farms had been established during the outbreak investigation on farm 27. Not easy to use.

Spoligotyping

Spoligotyping was performed on 26 isolates from 16 farms resulting in 9 different spoligotypes. Of these, two were each associated with unrelated outbreaks on two farms(SP1, SP9), three were found in 3 unrelated outbreaks (SP2, SP4, SP8) while four spoligotypes were associated with individual outbreaks (SP3, SP5, SP6, SP7). Spoligotype SP7 was found along with SP2 on the same farm (farm 9).

For a total of eleven herds (farms 3, 7, 9, 10, 11, 19, 22, 26, 27, 29, 38) more than one *M. bovis* isolate was available for analysis. As shown in Table 2, all isolates originating from the same farm, namely farms 10, 19, 22, 27, 29 and 38 were found to belong to the same genotype as determined by one or more typing methods. In contrast, the BTB outbreaks on farms 3, 7, 9, 11 and 26 were associated with two genetically different *M. bovis* strains. Farms 26 and 27 were found to have a common M. bovis genotype, as identified by both PGRS and IS*6110*. Traditional outbreak investigation revealed that infected cattle had been sold from farm 27 to 26.

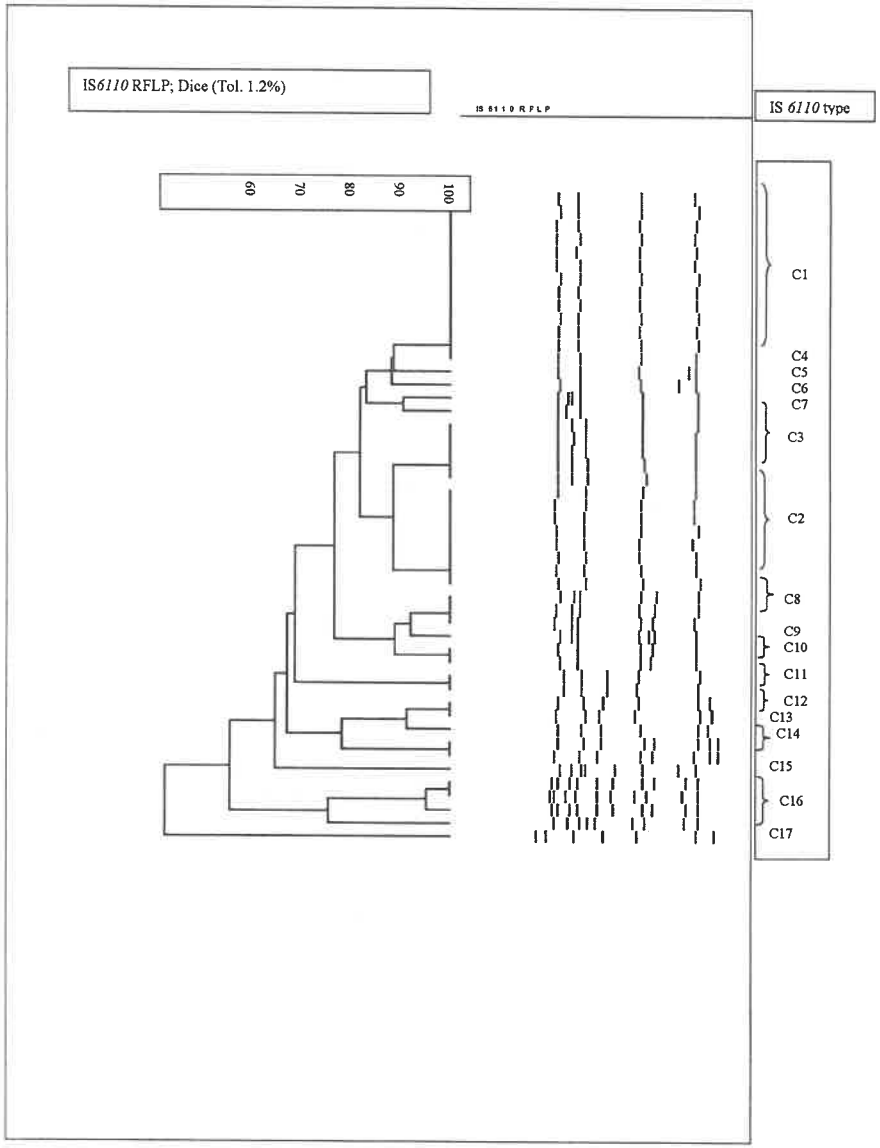

Fig. 2. IS*6110* dendogram of 50 *M. bovis* isolates

DISCUSSION

The present study is the first to investigate DNA polymorphism of *M. bovis* in cattle in South Africa, where bovine tuberculosis continues to play an important role as disease of livestock.Three of the most commonly used genetic markers IS*6110*, PGRS and spoligotyping were applied to determine the genetic diversity among 58 *M. bovis* field isolates cultured from routine diagnostic cases . The results summarised in Tables 1 and 2 demonstrate that PGRS RFLP was the most discriminatory method as it was able to discriminate between all 18 epidemiologically unrelated outbreaks investigated with this method. Furthermore three of the outbreaks (farms 3, 11 and 26) were found to be associated with 2 genetically different *M. bovis* strains, bringing the total of PGRS types identified to 20. Despite its high discriminatory power PGRS typing is cumbersome as the complexity of its banding patterns requires in many cases that RFLP data are analysed visually (Cousins et al. 1998b). Spoligotyping and IS*6110* RFLP both support computer-assisted analysis of genotyping data but spoligotyping on its own was not found to provide satisfactory differentiation of strains in this study. Through combination with IS*6110* RFLP the number of genotypes identified was increased from nine to thirteen (Table 1). Overall IS*6110* RFLP can be considered the typing method of choice to detect genetic differences in isolates with multiple copies of this insertion sequence (Skuce & Neill, 2001; Cousins 1998a). IS*6110* RFLP appeared to be highly discriminatory for all *M. bovis* strains which contained more than three copies of the IS sequence (Fig. 2). However, fifty percent of the isolates subjected to this typing method clustered with one of two *M. bovis* strains containing only 2 copies of *IS*6110, hence limiting the value of this probe. It was possible to increase the level of discrimination by using additional PGRS typing as recommended by Cousins et al. (1998b).

Two stage genotyping has also proven useful in studies on *M. tuberculosis* isolates with low copy numbers of IS*6110* (Bauer 1999, Gillespie 2000, Rasolofo-Razanamparany 2001). The use of different markers has the additional advantage of confirming co-existence of unrelated strains in the same herd beyond doubt, as recommended by Cousins et al. 1998b. In our investigation both the *IS*6110 and PGRS RFLP typing methods identified farm 27 as the point source of infection for farm 26 which had purchased cattle from farm 27 prior to detecting the disease.

Multiple isolates from a total of eleven herds (Table 1) were available for analysis and served to confirm reproducibility of banding patterns and to test for possible co-existence of different *M. bovis* strains in the same herd. Five of the herds were found to be co-infected with two distinct genotypes. Multi-genotype infections may not be a rare event, especially in countries where BTB occurs at a prevalence of of > 1% (Serraino et al. 1999). Costello et al. 1999 found that 10% of cattle herds examined in the Republic of Ireland harbored more than one strain. We were unable to reliably estimate the percentage of herds with multiple strain involvement due to the small number of outbreaks examined However, the fact that such events were detected in the small sample may either suggest a fairly high frequency of outbreaks with multiple sources of infection probably due to purchase of infected animals (Skuce et al. 1994, Neill et al. 1994) or, alternatively, to persistence of an "old" infection with evolution of the *M. bovis* strain (Milian-Suazo et al. 2002). Both scenarios appear plausible in the South African context. Despite early successes in the reduction of BTB in South Africa to below 0.1% in commercial cattle herds, the disease has more recently been detected in eight of the nine provinces (Michel, unpublished data). This may partially be ascribed to the decline in annual tuberculin testing of cattle herds allowing uncontrolled progression of the disease in infected but undiagnosed herds. Eradication of outbreaks is, however, further complicated through the lack of a market-related compensation scheme for infected cattle, resulting in poor collaboration from farmers and delays of more than one year in the slaughtering of infected animals are not uncommon (Connoway, Walters, pers. comm.) Movement of infected animals from such herds is more likely to happen the longer an outbreak persists, regardless of whether livestock owners are aware of the presence of the disease in their herds or not.

The genetic diversity detected among *M. bovis* isolates in this study appears to be higher compared to most studies conducted in other countries. Genotyping of 233 *M. bovis* isolates from

cattle in the Republic of Ireland yielded 17 spoligotypes and 15 IS*6110* types (Costello 1999) and Skuce et al. (1994) found ten different IS*6110* types among 109 cattle isolates. Spoligotyping of 1349 *M. bovis* isolates in France identified 161 spoligotypes (Haddad 2001). It is possible that the importation of cattle from many different countries during and after colonisation of South Africa (Cousins et al. 2004) contributed to the genetic variation of *M. bovis* in this country. Despite a significant reduction in the prevalence, BTB was never fully eradicated from this country and may have evolved in sufficient diversity to account for at least the degree of genetic polymorphism observed in this limited study.

In conclusion we believe that IS*6110* and PGRS will be useful markers to study interbovine and interspecies BTB transmission in South Africa. At the same time it will be important to evaluate the newer technique of VNTR typing as it has been reported to reveal the highest level of polymorphism between isolates (Haddad et al. 2004).

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Source of M. bovis: Spread from new outbreaks or from old outbreaks (re-activated outbreaks)

CLINICAL UTILITY OF A PCR-BASED TESTING STRATEGY FOR THE DIAGNOSIS OF *MYCOBACTERIUM BOVIS* INFECTION IN CATTLE

J. Godfroid[1,2], M. Govaerts[1] & K. Walravens[1]

ABSTRACT

The aim of this study was to define and validate a rapid and reliable PCR-based testing strategy, that would either confirm the presence either certify the absence of *M. bovis* in a given sample. From 2002 to 2004, 681 tissue samples were submitted to the Belgian Reference Laboratory for Bovine Tuberculosis. These samples (taken at the abattoir) originated from animals suspected to be infected with *M. bovis*, i.e. skin test reactors, animals in contact with *M. bovis* infected animals or showing suspect lesions during meat inspection. A positive *M. bovis* culture, identified within 6 Weeks post-inoculation of Coletsos® slopes (Biorad), was the gold standard. 16S RNA PCR (Amplicor®, Roche) was carried out on DNA extracted either at Day 1 from tissue samples [together with Ziehl-Neelsen staining (ZN) and Macroscopic Detection of Tuberculosis Lesions (MDTL)], or at Week 2 from Coletsos inoculated slopes. In 151 culture positive samples, PCR performed at Day 1, appeared to be a reliable tool compared to ZN or MDTL (sensitivity of 75%, 72% and 83% respectively). A second PCR performed on 2-Week old bacterial cultures increased the cumulative sensitivity to 93%. All *M. bovis* infected herds (n=38) were detected by PCR performed directly on tissue samples at Day 1. Specificity of the PCR was assessed on 174 culture negative samples originating from tuberculosis free herds: all were negative. These results re-emphasize the importance of MDTL and suggest that PCR is a reliable tool for tuberculosis herd certification. Although bacteriology at 6 Weeks post-inoculation remains the gold standard to certify the absence of *M. bovis* in a given sample, for 129 culture negative samples originating from animals in *M. bovis* infected herds, 8 were PCR positive.

INTRODUCTION

The search for a rapid, accurate, yet inexpensive test for the diagnosis of active human tuberculosis (HTB due to *Mycobacterium tuberculosis*), begun almost a century ago (1), has become the equivalent of the search for the holy grail. At present the most widely used rapid test is the direct microscopic examination of a smear of sputum for acid-fast bacilli (AFB smear). However the preparation and reading of the smear are time consuming and detect only 40-80% (2) of pulmonary HTB cases, and only the more advanced cases (3). Diagnosis of patients at an earlier stage, while still smear negative, would be advantageous because they are less contagious (4, 5) and have lower morbidity and mortality (6). Mycobacterial cultures are highly sensitive, but take at least 2 wk or longer if solid media are used (often 4-8 weeks), and culture facilities are not available in many countries. Recently developed nucleic acid amplification techniques have specificity of more than 95%, and are more than 95% sensitive in smear-positive specimens (7-9). However these tests are expensive, require sophisticated technology, and have sensitivity of only 50-71% in patients with smear negative active HTB (8), hence the clinical setting in which a rapid diagnostic test other than the AFB smear is most needed.

As far as bovine tuberculosis (BTB, due to *Mycobacterium bovis*) is concerned, in most countries worldwide, control or eradication programs are implemented. Depending on the epidemiological situation, assessment of the herd tuberculosis status is usually performed either by using the skin test (simple or comparative) either during routine meat inspection at the abattoir (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2004;

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http://www.oie.int/eng/normes/mmanual/A_summry.htm). Control and eradication programs are based on the timely (i.e. early) detection of the infection, before shedding occurs in order to avoid contagion. Confirmation of tuberculosis relies on the isolation of *M. bovis*. In the European Union (EU), eradication programs are implemented (ECC Directive 64/432). The target for each Member State (MS) is to be able to document that 99.8% of the national herds are free from *M. bovis* infection in order to gain the "Tuberculosis Officially Free Status" (TbOF). Once this status is obtained, trade (i.e. safe and free circulation of the animals in the EU territory) is allowed and testing for trade purposes does not have to take place. Should a MS loose its status, then trade is restricted and tuberculosis testing prior animal movements has to be enforced. This means that economical implications linked to the TbOF status are extremely important.

As for HTB, the search for a rapid, accurate, yet inexpensive test for the early diagnosis of BTB has become the equivalent of the search for the holy grail. In the HTB field, a lot of emphasis is put on the accuracy and utility of commercially available amplification assays for the early detection of HTB (10). Surprisingly, contrary to the situation for HTB, to date, the documented information on the definition of a testing strategy for BTB in the context of control or eradication programs is very scarce (11, 12) although PCR techniques have been described in domestic animals (13, 14) as in wildlife (15).

The aim of this study was to define and validate a rapid and reliable PCR-based testing strategy, that would either confirm the presence either certify the absence of *M. bovis* in a given sample.

MATERIALS AND METHODS

Samples

From 2002 to 2004, 681 tissue samples were submitted to the Belgian Reference Laboratory for Bovine Tuberculosis (Veterinary and Agrochemical research Center). These samples (taken at the abattoir) originated from animals suspected to be infected with *M. bovis*, i.e. skin test reactors, animals in contact with *M. bovis* infected animals or showing suspect lesions during meat inspection.

Case definition and testing strategy

A positive *M. bovis* culture, identified within 6 Weeks post-inoculation of Coletsos® slopes (Biorad), was the gold standard.

16S RNA PCR (Amplicor®, Roche) was carried out on DNA extracted either at Day 1 from tissue samples [together with Ziehl-Neelsen staining (ZN) and Macroscopic Detection of Tuberculosis Lesions (MDTL)], or at Week 2 from Coletsos® inoculated slopes.

Microbiologic tests

Samples from Tb suspected animals were homogenized with sterile physiologic water, and decontaminated with 5% Oxalic acid for 20-30 min at 37°C, centrifuged at 3,500 rpm (1,068 x g) for 20 min, and cultured onto Coletsos® medium (Biorad, Belgium) at 37°C. The isolates were identified as *M. bovis* by staining for acid-alcohol fastness, preferential grow at 37°C/42°C, Nitrate reductase (Beckton Dickinson, Belgium) and Tiophene-2-carboxilic acid hydrazide (TCH) assays (Biomérieux, Belgium).

Nucleic acid amplification and detection techniques

Homogenised organ samples were heat inactivated before extracting DNA using High Pure PCR Template Preparation Kit (Roche, Germany) following instruction manual. PCR test was than performed with the Roche Amplicor Mycobacterium test (Amplicor MTB), a qualitative *in vitro* diagnostic test for the detection of members of the Mycobacterium tuberculosis complex (i.e. *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum and Mycobacterium microti*). The test was performed according to the manufacturer's instructions (Roche Diagnostics, Germany).

Specimens and controls (three negative and one positive) were amplified in a Biorad iCylcler thermal cycler. In addition a sequence of plasmid DNA with primer binding regions identical to those of the M. tuberculosis sequence was introduced into each reaction mixture and coamplified with the target DNA to provide an internal control of the PCR reaction. Carryover contamination was prevented by incorporation of dUTP in place of dTTP in the amplification reaction, and utilization of uracil-N-glycosylase (AmpErase, Roche) to cleave any amplicon carried over from previous reactions.

Detection of mycobacteria of the *M. tuberculosis* complex was accomplished by hybridization of the biotin-labeled amplicon to probe-coated microwell plates, and addition of an avidine horseradish peroxidase conjugate and a tetramethylbenzidine substrate. The optical density at 450 nm was measured using an automated microwell plate reader. Specimens with an absorbance greater than 0.35, were interpreted as positive, irregardless of the internal control result. Specimens with an absorbance less than 0.35, and an internal control absorbance greater than 0.35, were classified as negative. Specimens with an absorbance less than 0.35, and an internal control absorbance less than 0.35, were classified as uninterpretable.

All technicians who carried out the PCR were blind to the final diagnosis.

RESULTS

In 151 culture positive samples, PCR performed at Day 1, appeared to be a reliable tool compared to ZN or MDTL (sensitivity of 75%, 72% and 83% respectively).

A second PCR performed on 2-Week old bacterial cultures increased the cumulative sensitivity to 93%. All *M. bovis* infected herds (n=38) were detected by PCR performed directly on tissue samples at Day 1.

Specificity of the PCR was assessed on 174 culture negative samples originating from tuberculosis free herds: all were negative.

DISCUSSION

This study demonstrates that accurate and rapid diagnosis of BTB remains difficult, and there is still no single ideal test. The nucleic acid amplification technique used had excellent specificity, but only moderate sensitivity, particularly for animals showing no gross lesions in the lungs and/or associated lymphnodes, compatible with BTB. A positive PCR would indicate disease with a very high degree of certainty, but a negative result would be less helpful in situations with greater likelihood of disease (when gross lesions are present in animals originating from a BTB infected herd in our study). In the context of BTB eradication program, although the definitive diagnosis depends on the isolation of *M. bovis*, the emphasis is not put on the positive predictive value of the PCR but on its negative predictive value (which is of concern given the relative poor sensitivity of the test). This is why, other criteria or tests have to be used in order to provide additional information to guide managers of the eradication programs.

We therefore propose the following strategy:

- If typical lesions are present in the lung and lung associated lymphnodes:
  MDTL is the most sensitive detection method and thus other techniques will at best identify the same samples as positive. Therefore, we suggest that in cases animals have been detected by a positive skin test, in the presence of typical gross lesions (seen in the abattoir or in the laboratory) further confirmation via culture or PCR is not needed for diagnostic *per se* (although it may be required by law). However, it is advised to isolate at least one strain per herd for further molecular typing (16). The sensitivity of this method is 83%. Conversely, in the context of an eradication program, absence of gross lesion does not mean absence of infection. Indeed, in our study 17% of BTB infected animals did not develop gross lesion, because of the early detection strategy.

Macroscopic detection of lesions

- If typical lesions are absent in the lung and lung associated lymphnodes:
  Culture should be performed. On Week 2 : PCR should be performed on DNA extracted from Coletsos® inoculated slopes.

The cumulative sensitivity of the MDTL performed at day 1 and the PCR performed at Week 2 is 93 %. This means that for 7% of the BTB samples, a positive *M. bovis* culture, will only be identified 6 Weeks post-inoculation of Coletsos® slopes.

To summarize, 83% of the BTB positive samples will be identified at day 1; 93 % at week 2 and 100% at week 6. As a consequence, if BTB is suspected in a herd, and in the absence of gross lesions in animals that where identified at risk (skin test reactors and animals in contact with *M. bovis* infected animals), it is virtually impossible to certify the absence of BTB for a period of 6 weeks following the start of *M. bovis* culture.

These results re-emphasize the importance of MDTL and suggest that PCR is a reliable tool for tuberculosis herd certification that is part of a testing strategy aimed at certifying the absence of BTB.

Nowadays, it appears that mycobacterial cultures are still the most sensitive of currently available tests for the diagnosis of HTB and BTB. However, even cultures may be false negative. Thus, although bacteriology at 6 Weeks post-inoculation remains the gold standard to certify the absence of *M. bovis* in a given sample, in our study, for 129 culture negative samples originating from animals in *M. bovis* infected herds, 8 were PCR positive. Given the specificity of this PCR, a positive PCR result, even in the absence of positive culture, should be considered as a true BTB case.

Scrofula : human tb - originates from milk.

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Wildlife:

Is these a spillover or a reservoir? In UK there is a reservoir - it is not deer. These are a spill over. The badger is a reservoir - can reinfect cattle. Skin test positive cattle may be paratuberculosis (Johne's disease) +ve.

Sensitivity:

PCR sensitivity is only about 75%. The specificity is 91%.

Meat inspection:

Only shows between 29 and 45% of lesions cases. The retropharyngeal ln. is usually infected in cattle. This ln. should be checked.

GENETIC ANALYSIS OF SAT-1 TYPE FOOT AND MOUTH DISEASE OUTBREAKS IN SOUTHERN AFRICA – A HISTORICAL OVERVIEW

W. Vosloo[1,2], A.D.S. Bastos[1,3] & C.I. Boshoff[1,4]

ABSTRACT

In areas where foot and mouth disease (FMD) is endemic in wildlife hosts, such as the Kruger National Park (KNP) in South Africa, control measures are in place to ensure that wildlife does not come into close contact with domestic animals and the latter are vaccinated as additional preventive measure. In southern Africa the African buffalo (*Syncerus caffer*) are persistently infected with the 3 South African Territories (SAT) type viruses and they can potentially infect other susceptible wild and domestic cloven-hoofed animals when in close contact. Antelope in turn spread infection easily due to their ability to cross fences.

In South Africa several SAT-1 outbreaks occurred nearly simultaneously in cattle and impala between 1971-1981. Phylogenetic analysis based on partial 1D gene nucleotide sequencing indicated that several of these outbreaks were linked. As buffalo-proof fences were in place at that time it is probable that disease spread from the intermediary impala antelope host to cattle in close proximity. Evidence was found for the involvement of viruses from a single KNP genotype in precipitating outbreaks in impala over a 10-year period. In addition, several unrelated outbreaks affecting cattle and impala occurred within a single year. Characterisation of outbreak strains from Botswana similarly revealed that a single genotype affected different species over a 10-year period and that transboundary spread of SAT-1 virus occurred on at least one occasion. This retrospective analysis of outbreak strains has clearly demonstrated that FMD control policies that address the role of antelope as intermediaries in disease transmission are crucial as these wildlife species play an important role in disease dissemination.

INTRODUCTION

Foot and mouth disease (FMD) is a vesicular disease that affects over 70 cloven-hoofed species (Hedger, 1981). Although it depresses productivity in high producing farming systems and has a high morbidity, the mortality in adult animals is normally low. However, the economic effects can be devastating. FMD is endemic in Africa and the epidemiology of the disease is more complicated than in other parts of the world. In addition to having endemic infection by six of the seven serotypes of FMD virus, serotype distribution differs between regions and intratypic variants within serotypes occur (Vosloo, Bastos, Sangare, Hargreaves & Thomson, 2002). The three South African Territories (SAT) serotypes are prevalent in southern Africa. Historically it has been shown that most outbreaks in the region are caused by SAT-2, followed by SAT-1 and lastly by SAT-3 viruses (Thomson, 1994). Of the five outbreaks that occurred outside the Kruger National Park (KNP) in South Africa since 2000, the first was shown to be an exotic introduction of the Pan-Asian O virus, three were caused by SAT-2 and one by SAT-1. The latter four outbreaks are believed to have been precipitated by buffalo that were able to move out of the game park when fences were compromised (Vosloo, Boshoff, Dwarka & Bastos, 2002).

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The SAT type viruses are maintained by the African buffalo (*Syncerus caffer*) (Thomson, 1994) and evidence has shown these animals to be the source of infection for susceptible livestock in close proximity to the KNP where infected buffalo occur. Infection in buffalo is sub-clinical and after infection approximately 60% of buffalo become carriers and FMD virus can be maintained for periods of up to 24 years in an isolated herd (Condy, Hedger, Hamblin & Barnett, 1985). Outbreaks of FMD occur regularly in impala (*Aepyceros melampus*) in the KNP and phylogenetic analysis has unequivocally shown buffalo to be the source of infection for impala (Bastos, Boshoff, Keet, Bengis & Thomson, 2000). Impala are highly susceptible to FMD virus. The role of impala in the epidemiology of FMD is unclear, but since they do not become carriers of the disease, it is accepted that in South Africa at least, they are not important in the epidemiology of FMD (Vosloo & Thomson, 2004). However, during the acute stage of infection, infected animals excrete virus in all body secretions and can infect other susceptible animals.

South Africa obtained zoned disease-free status without vaccination from the World Organisation for Animal Health in 1995 by dividing the country into different zones and separating the endemic (KNP) from the free zone by a buffer zone where vaccination of livestock is allowed. The game fence surrounding the KNP has also been upgraded. These measures have proven to be mostly successful in controlling FMD, but prior to that numerous outbreaks occurred outside the KNP in domestic animals (Fig. 1). On several occasions, clinical cases were observed in cattle and impala simultaneously, and although they were shown to be of the same serotype, it was not proven whether these outbreaks were related.

In this paper, historical SAT-1 isolates obtained from impala and cattle between 1971 to 1981 in regions within and surrounding the KNP were genetically compared to 1D gene sequences of southern African buffalo isolates in order to determine possible routes of disease dissemination.

MATERIALS AND METHODS

Viruses included in the study

A total of 57 SAT-1 virus isolates from cattle, impala antelope, buffalo and a single kudu antelope (*Tragelaphus strepsiceros*) obtained between 1948 and 1998 from 8 countries in southern Africa were included in this study (Table 1, Fig. 1). Historical outbreak isolates were obtained from the FMD World Reference Laboratory at the Institute for Animal Health, Pirbright, United Kingdom and were propagated on IBRS-2 cells to increase virus titres prior to RNA extraction.

Table 1. List of the SAT-1 viruses included in this study for which 35 new sequences were generated and the remaining 22 sourced from published reports. The date of collection, geographical origin and species of origin are indicated.

Strain designation	Sampling date	Country	Place of origin	Species of Origin
*#BEC/1/48	1948	Botswana	NA	Cattle
*#SAR/13/61	Mar, 1961	South Africa	Potgietersrus	Cattle
*$BOT/1/68	Jan, 1968	Botswana	Satau	Cattle
*#SAR/2/71	Aug, 1971	South Africa	Elmboog, Komatipoort	Cattle
*#SAR/3/71	Aug, 1971	South Africa	Sugar Station, Komatipoort	Cattle
*#SAR/8/71	Apr, 1971	South Africa	Sugar Station, Komatipoort	Impala
*#SAR/10/71	Feb, 1971	South Africa	Sugar Station, Komatipoort	Impala
*#SAR/11/71	Mar, 1971	South Africa	Sugar Station, Komatipoort	Impala
*#SAR/12/71	Aug, 1971	South Africa	Trollip Farm, Sabie Sand	Impala
*#SAR/1/73	Oct, 1973	South Africa	Area 3, Kruger NP	Impala
*#SAR/2/73	Oct, 1973	South Africa	Area 3, Kruger NP	Impala
*#SAR/4/74	Apr, 1974	South Africa	Letaba	Cattle
*#SAR/23/74	Sep, 1974	South Africa	Skukuza, Kruger NP	Impala

Strain designation	Sampling date	Country	Place of origin	Species of Origin
*#SAR/25/74	Sep, 1974	South Africa	Skukuza, Kruger NP	Impala
*#SAR/26/74	Sep, 1974	South Africa	Skukuza, Kruger NP	Impala
*#SAR/29/74	Sep, 1974	South Africa	Skukuza, Kruger NP	Impala
*#SAR/30/74	Sep, 1974	South Africa	Skukuza, Kruger NP	Impala
*#SAR/5/75	Feb, 1975	South Africa	Grootdraai, Hectorspruit	Cattle
*#SAR/6/75	Feb, 1975	South Africa	Grootdraai, Hectorspruit	Cattle
*#BOT/1/77	Oct, 1977	Botswana	Nokaneng	Cattle
*#BOT/17/77	1977	Botswana	30km SW of Habu	Kudu
*$BOT/24/77	1977	Botswana	Habu	Cattle
*$MOZ/3/77	Dec, 1977	Mozambique	Choque	Cattle
*#SAR/4/79	Oct, 1979	South Africa	Sibasa, Venda	Cattle
*#SAR/17/80	Jul, 1980	South Africa	Matiani dip tank	Cattle
*#SAR/4/81	Jun, 1981	South Africa	Tshokwane, Kruger NP	Impala
*#SAR/5/81	Jun, 1981	South Africa	Tshokwane, Kruger NP	Impala
*$SAR/9/81	Oct, 1981	South Africa	Pafuri, Kruger NP	Impala
§MAL/1/85	1985	Malawi	Kasungu NP	Buffalo
#KNP/3/86	Mar, 1986	South Africa	Tshokwane, Kruger NP	Buffalo
#KNP/6/86	Mar, 1986	South Africa	Tshokwane, Kruger NP	Buffalo
#KNP/10/86	Nov, 1986	South Africa	Punda Maria, Kruger NP	Buffalo
#KNP/1/87	Nov, 1986	South Africa	Mooiplaas, Kruger NP	Buffalo
*$ZIM/3/88	Jul, 1988	Zimbabwe	Hwange NP	Buffalo
#KNP/3/89	Jun, 1989	South Africa	Rietpan, Kruger NP	Buffalo
#KNP/4/89	1989	South Africa	Mala Mala, Kruger NP	Buffalo
#KNP/8/89	Dec, 1989	South Africa	Meseldam, Kruger NP	Buffalo
#KNP/15/89	1989	South Africa	Meseldam, Kruger NP	Buffalo
#KNP/20/89	Oct, 1989	South Africa	Kwa Mfamebto, Kruger NP	Buffalo
#KNP/26/89	Oct, 1989	South Africa	Kwa Mfamebto, Kruger NP	Buffalo
#KNP/39/89	Oct, 1989	South Africa	Ngwenyeni, Kruger NP	Buffalo
§KNP/196/91	Jun, 1991	South Africa	Mbyamiti, Kruger NP	Buffalo
§ZIM/2/91	Oct, 1991	Zimbabwe	Bumi Hills NR	Buffalo
§ZAM/2/93	May, 1993	Zambia	Nanzhila, Kafue NP	Buffalo
*$ZIM/2/94	Oct, 1994	Zimbabwe	Sinamatela, Hwange NP	Buffalo
§KNP/14/95	Nov, 1995	South Africa	Mondsweni, Kruger NP	Buffalo
§KNP/17/95	Nov, 1995	South Africa	Mondsweni, Kruger NP	Buffalo
*$ZIM/3/95	Jun, 1995	Zimbabwe	Sengwa, Lake Kariba	Buffalo
§KNP/2/96	Sep, 1996	South Africa	Mala Mala, Kruger NP	Buffalo
§ZAM/18/96	Jun, 1996	Zambia	Nanzhila, Kafue NP	Buffalo
§UGA/1/97	1997	Uganda	Hamukungu, Queen Elizabeth NP	Buffalo
§BOT/2/98	Jul, 1998	Botswana	Nxaraga	Buffalo
§BOT/8/98	Jul, 1998	Botswana	Nxaraga	Buffalo
§BOT/14/98	Jul, 1998	Botswana	Nwaraga	Buffalo
§BOT/25/98	Jul, 1998	Botswana	Vumbura	Buffalo
§BOT/37/98	Jul, 1998	Botswana	Vumbura	Buffalo
§NAM/272/98	Aug, 1998	Namibia	Mamili NP	Buffalo

NA: Not available
* Isolates supplied by the WRL (Pirbright)
# Sequence determined for this study
$ Bastos, 1998
§ Bastos, Haydon, Forsberg, Knowles, Anderson, Bengis, Nel & Thomson, 2001

Genomic characterisation

RNA was extracted from cell culture isolates and cDNA synthesized. The C-terminal half of the VP1 gene was PCR amplified, and products of the expected amplicon size were excised from agarose and purified. Sequences were determined manually using Sequenase version 2.0 (USB) in the presence of 10% DMSO (Bastos, 1998).

Phylogenetic analysis

The 35 nucleotide sequences generated in this study were complemented with 22 additional sequences. Modeltest 3.0 (Posada & Crandall, 1998) was used to identify the model of sequence evolution. A VP1 gene tree was subsequently constructed in MEGA 2.1 (Kumar, Tamura, Jakobsen & Nei, 2001) using the minimum evolution algorithm, Tamura-Nei substitution correction model and a gamma distribution shape parameter of 0.95. Nodal support was obtained following 10 000 bootstrap replications.

RESULTS

Phylogenetic analysis of the 57 SAT-1 isolates included in this study demonstrated the three main topotypes previously described for SAT-1 viruses in southern Africa (Fig. 2; Bastos, Haydon, Forsberg, Knowles, Anderson, Bengis, Nel & Thomson, 2001). Isolates from all species collected in the KNP and its immediate surroundings, as well as a single isolate from Mozambique clustered in topotype 1. Viruses from buffalo, cattle and one kudu from Botswana, Namibia and western Zimbabwe clustered in topotype 2, while topotype 3 consisted of buffalo isolates from Malawi, Zambia and northern Zimbabwe. Most outbreak viruses clustered within the topotype distributional range of the natural buffalo hosts, indicating that outbreaks were precipitated by contact between cattle and wildlife occurring in close proximity. However, an outbreak that occurred in cattle close to Potgietersrus during 1961 in South Africa, clustered in topotype 2, indicating that the origin of this outbreak was from outside the borders of South Africa (Figs. 1 and 2).

Indications of disease spreading among countries and species were detected between a cattle isolate obtained in Choque, Mozambique during 1977 which grouped with an impala isolate from Pafuri in the north of the KNP during 1981 and a 1979 cattle virus from Sibasa, South Africa (Figs. 1 and 2).

Several outbreaks in South Africa involved cattle and impala and phylogenetic analysis of the partial 1D sequences indicated that isolates from impala obtained close to Komatipoort in 1971 had nearly 100% sequence identity over the region sequenced to cattle viruses obtained in close proximity (Figs. 1 and 2). The impala viruses were isolated between February and April 1971, whilst the disease was only discovered in cattle in August of the same year. A virus with nearly 100% sequence identity was also found in August in impala at Sabie Sand, a significant distance away (Figs. 1 and 2).

During 1981 an outbreak was observed in impala near Tshokwane in the KNP that clustered with impala viruses from the 1971 outbreak. The bootstrap value of 79% and 7% maximum nucleotide difference confirmed that the viruses belonged to one genotype that may have circulated in the field for at least ten years.

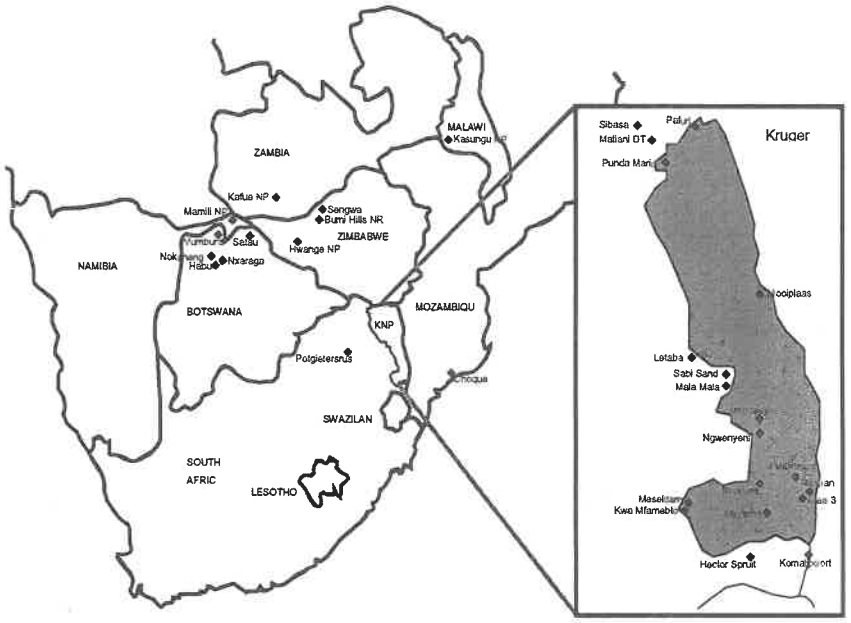

Figure 1. Map of southern Africa indicating the geographic origin of the SAT-1 isolates included in this study

The results provide evidence that disease spread from impala in Area 3 of the KNP in 1973 to cattle in Letaba six months later (Figs. 1 and 2). This disease episode was unrelated to the 1971 outbreak, demonstrated by the 10% nucleotide sequence differences and a lack of bootstrap support. A separate disease incidence, again indicating possible spread from impala to cattle was observed during 1974-1975 but this extended outbreak was unrelated to any of the previously described epizootics (Fig. 2).

Only one outbreak in cattle could be directly linked to buffalo isolates from the KNP. All other buffalo viruses from the KNP grouped within topotype 1, but could not be linked to any of the outbreaks occurring in South Africa between 1971 and 1981.

Interspecies transmission was demonstrated in Botswana during 1977, where an isolate from cattle in Nokaneng (BOT1/77) had 100% sequence identity to another from a kudu antelope in Habu (BOT/17/77) over the 1D region sequenced (Figs. 1 and 2). Unfortunately, due to a lack of information, it is not possible to determine which species was initially infected. The relationship between isolates made in Botswana between 1968 and 1977 (9-10% nucleotide difference) indicated that viruses from the same genotype caused outbreaks in cattle in that country.

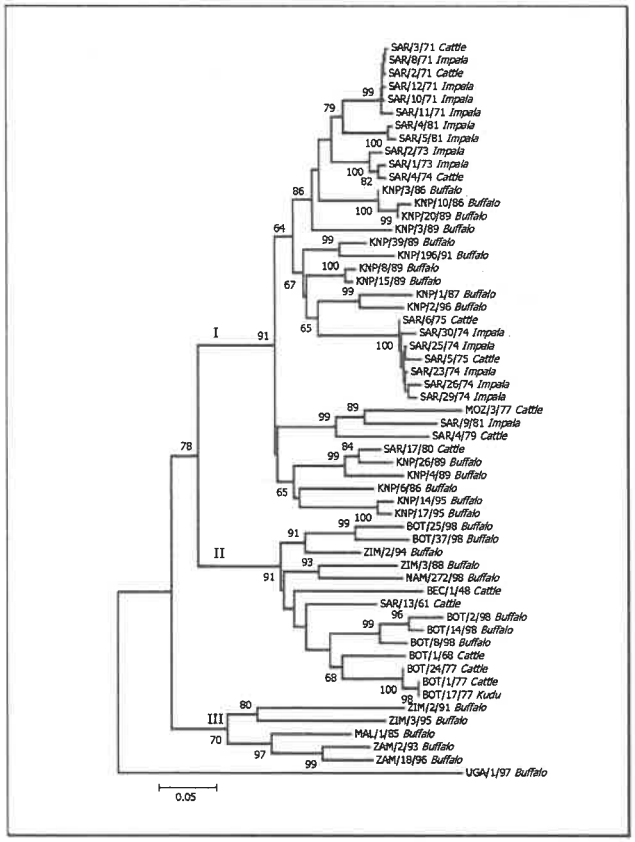

Figure 2. Minimum evolution tree indicating the phylogenetic relationship of the SAT-1 isolates obtained from various species in southern Africa between 1948 and 1998. The three main topotypes previously identified for this serotype are indicated by I-III and clusters supported by a bootstrap of >60% are shown.

DISCUSSION

Outbreaks in impala in the endemically infected KNP are a frequent occurrence and it is important to investigate the possible role that wildlife have in disseminating FMD to domestic animals. This can only be assessed retrospectively as outbreaks in cattle in South Africa are presently rare. Although circumstantial evidence indicates that historical outbreaks in cattle and impala might be linked, the use of nucleotide sequencing in this study has shown unequivocally that in many instances this was in fact the case. Five outbreaks occurred in impala between 1971 and 1981 of which four could be directly linked to cattle. One outbreak in cattle on the border of the KNP grouped with buffalo viruses occurring in the Park. This clearly demonstrates the potential for

? * Kudu also may play a role (Zimbabwe)

wildlife to infect domestic animals and should be regarded in FMD control strategies where this interface occurs.

For three of the epizootics, disease was reported in cattle 5-6 months after the first impala isolates were made, confirming the direction of spread to be from wildlife to cattle. However, it is also possible for livestock to transmit disease to wildlife (Anderson, Foggin, Atkinson, Sorenson, Madekurozva & Nqindi, 1993; Hedger, 1976; Thomson, Bengis, Esterhuysen & Pini, 1984). Due to a deficiency in historical surveillance information between 1977 and 1981, it is possible that disease spread from cattle to wildlife. However, as impala in KNP become infected by close contact with buffalo and are known to act as intermediaries between buffalo and cattle (Hargreaves, Foggin, Anderson, Bastos, Thomson, Ferris & Knowles, 2004; Sutmoller, Thomson, Hargreaves, Foggin & Anderson, 2000) it is more likely the 1977 and 1979 cattle outbreaks were due to multiple wildlife-cattle transmission events involving the same genotype.

Evidence for more than one disease episode caused by SAT-1 within a short time period in impala in the KNP was confirmed. During 1973 and 1974 two unrelated epizootics occurred in the KNP in the same region of the Park, while two episodes also occurred during 1981, but in the centre and north of the Park.

The data obtained in this study point to the possibility of long-term circulation of an SAT-1 genotype within impala herds over a 10 year period. This has also been described for SAT-2 where identical viruses have recurred in impala 6-18 months after the original outbreak (Keet, Hunter, Bengis, Bastos & Thomson, 1996; Vosloo, Knowles & Thomson, 1992). This mechanism of persistence is currently not known, as it is not clear how the virus survives inter-epidemic periods or whether the same virus had been transmitted on more than one occasion from buffalo to impala. Impala antelope do not seem to become persistently infected with FMD virus and serological evidence indicates that an immune response is transient and the numbers of sero-positive animals decreases progressively after an outbreak until all animals are negative (Anderson, Anderson, Doughty & Drevmo, 1975; Hedger, Condy & Golding, 1972; Vosloo & Thomson, 2004).

Although most of the outbreaks that involved different species were geographically linked, the outbreaks that occurred during 1973–74 in impala and cattle were significant distances from each other and could indicate that either the outbreak in impala was more widespread than recorded or significant wildlife movement happened at the time.

More than 90% of the SAT-1 outbreaks in impala occurred between June and November, coinciding with the time that buffalo calves become susceptible to infection and would be excreting virus in all secretions (Gainaru, Thomson, Bengis, Esterhuysen, Bruce & Pini, 1986). The same was previously found for SAT-2 outbreaks in impala (Bastos *et al.*, 2000) giving credence to the notion that buffalo are normally the source of infection to other species in the KNP.

Wildlife has the potential to infect domestic animals and so threaten a country's food security and agricultural exports. Antelope can furthermore cross fences and boundaries that may control movement of livestock. These factors should be considered in regions where wildlife occurs and has implications for vast tracts of sub-Saharan Africa. Although the role of buffalo in the epidemiology of FMD has been emphasised in the past, this study clearly indicates that other wildlife species play an important intermediary role in disseminating disease.

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POSTERS

PREVALENCE OF HYPODERMOSIS IN SOUTHERN PUNJAB (PAKISTAN)*

Zafar Iqbal[1], M. Nisar Khan, M. Sohail Sajid & M. Anwar

Prevalence of hypodermosis in cattle and buffaloes was recorded in south Punjab (Pakistan). For this purpose, the animals selected on the basis of proportional allocation were categorized with respect to age, sex, area and breed for field study. In slaughter house prevalence, different organs were also examined for the presence of various larval stages of the *Hypoderma* species. In Dera Ghazi Khan, the month-wise slaughter house prevalence in cattle (51.00 %; 2295/4500) and buffaloes (8.13 %; 183/2250) was higher than field prevalence in cattle (28.91 %; 1301/4500) and buffaloes (5.11 %; 115/2250). Similar prevalence was reported in Rajan Pur with higher slaughter house prevalence in cattle (48.22 %) and buffaloes (7.64 %) than field prevalence (26.15 % and 4.40 %, respectively). Prevalence on the basis of month, age, area, sex and organ was also calculated. The third instars were collected and identified. The results indicated a higher prevalence of hypodermosis in cattle as compared to buffaloes examined in slaughter houses and field conditions in Dera Ghazi Khan and Rajanpur districts.

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EVALUATING THE ANTIBODY RESPONSE OF CATTLE TO THE NON-STRUCTURAL PROTEINS OF SAT TYPE FOOT-AND-MOUTH DISEASE VIRUS AND COMPARING THE GENETIC VARIATION OF THE GENES ENCODING THESE PROTEINS

O.C. Phiri[1], H.G. van Rensburg[1,2], B. Böhmer[1], L.T. Lekoana[1,3], F.F. Maree[1], M.J. Sorril[3], J. Theron[3], J.J. Esterhuysen[1], S. Maree[4] & W. Vosloo[1]

The epidemiology of foot and mouth disease (FMD) on the African continent is more complex than elsewhere. Six of the seven serotypes are present and especially the 3 SAT serotypes demonstrate more genetic variation than do the others. Antigenic differences between the different topotypes within serotypes also indicate that tailor-made vaccines will be needed to different regions of the continent. In southern Africa the SAT types predominate and FMD is maintained in the African buffalo (*Syncerus caffer*). Disease control relies on movement restrictions and vaccination in specified zones. Sensitive and specific serological assays are required to enable the detection of all seven FMDV serotypes and allow differentiation between vaccinated and convalescent infected animals. Whereas inactivated virus vaccines elicit antibodies primarily to the virus capsid, virus replication elicits additional antibodies directed against the non-structural proteins (NSPs) 2B, 2C and 3ABC and its components 3A, 3B and 3 AB. Detection of these NSPs antibodies is utilised to identify animals that have potentially been infected. Several commercial assays have been developed for the detection of antibodies to NSPs based on the European serotype 3ABC protein or components thereof and have been reported to be sensitive and specific. Cattle were infected with two SAT-1, two SAT-2 and one SAT-3 virus from different topotypes, serially bled and the antibody levels determined using four different commercially available kits. The duration of antibodies to the various viruses differed significantly and with only one SAT-1 strain was long-term presence of antibodies detected. The 3ABC polyprotein-coding region of SAT1, 2 and 3 field isolates were analysed and phylogenetically compared to the European serotypes. The SAT gene coding regions clustered separate from the other serotypes and indicated a possible need to develop tests specifically for the detection of antibodies to the NSPs of the SAT serotypes. The 3ABC polyprotein-coding region of SAT2/ZIM/7/83 was cloned and expressed in *Escherichia coli*, as well as in *Spodoptera frugiperda* cells by means of a recombinant baculovirus. The potential use of these expressed proteins will be investigated in a SAT type-specific NSP ELISA.

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PHYLOGENY, ANTIGENIC VARIATION AND EPITOPE PREDICTION OF THE CAPSID-CODING REGIONS OF THE SOUTH AFRICAN TERRITORIES (SAT) TYPES OF FOOT-AND-MOUTH DISEASE VIRUS

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Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals which is prevalent in Africa, Asia and South America and poses a threat to FMD-free countries. FMD virus (FMDV) exists as seven serologically distinct serotypes A, C, O, SAT (South African Territories) 1, 2, 3, and Asia 1 and serological typing has indicated that a number of antigenic variable subtypes are present within each serotype. FMD is endemic throughout Africa where six FMDV serotypes, with the exception of Asia-1, have been encountered. The SAT types, usually confined to sub-Saharan Africa, show marked genomic and antigenic variation, with the 1D-coding sequence appearing inherently more variable. This variation is geographically linked and tracing the origin of outbreaks is essential in establishing control measures. In addition, an effective vaccination program necessitates monitoring of emerging field isolates in relation to the current vaccines. Traditionally the *in vitro* neutralization test, using antisera against vaccine strains, is used to measure the antigenic relationship of field isolates. In recent years the development of molecular epidemiological techniques has offered the possibility of more detailed analyses of outbreak strains and sequencing databases have facilitated the study of FMD epidemiology. However, the current database for the SAT types is restricted mainly to the 1D-coding region, which comprises the major neutralizing epitopes. To address this inadequacy, the genetic variation within the P1 coding region of representative FMDV strains of the SAT types found in sub-Saharan Africa was analyzed and their antigenic relationships with reference strains from one topotype determined. For SAT-1 antigenic relationships within one topotype were acceptable for predicted vaccine protection, but between topotypes specific vaccine strains may be necessary. However, the antigenic variation within a single topotype of SAT-2 was sufficient to warrant more than one strain for protection or inclusion of viruses with broader antigenic spectrums. Hypervariable regions in the amino acid alignments were mapped to surface exposed epitopes using 3D reconstruction of the SAT capsid proteins 1A-1D based on published O data and good correlation between the structures were observed.

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PRELIMINARY RESULTS OF VALIDATION OF A FOOT-AND-MOUTH DISEASE ANTIBODY SCREENING SOLID-PHASE COMPETITION ELISA (SPCE) IN COMPARISON WITH THE LIQUID PHASE BLOCKING ELISA

O.C. Phiri[1], J.J. Esterhuysen[1] & W. Vosloo[1]

Foot and mouth disease (FMD) is an economically important disease which leads to trade restrictions and production losses. Countries free of the disease take severe measures to guarantee their disease-free status and diagnostic tests play an important role in ensuring safe trade of animals and animal products. Serological tests are often used as a fast and efficient way to determine the disease status of animals and therefore need to have a high sensitivity and specificity. A liquid-phase blocking ELISA (LPBE) has been used in the past to determine the FMD antibody status of animals, but international recommendation is that it should be replaced by a solid-phase competition ELISA (SPCE) which is an improved test for the classical European serotypes of FMD virus. Little is known about its performance with the 3 SAT serotypes that are prevalent in sub-Saharan Africa.

The SPCE for the serological detection of antibody to serotypes SAT-1 and SAT-2 of FMD virus was validated in cattle and titrated against known negative and positive sera for both serotypes. A comparison was made for serial dilutions to determine the optimal dilution for the assay. Optimum differentiation of positive and negative sera was obtained for sera used at a 1/10 dilution. A provisional cutoff point was established at 25 percentage inhibition (PI) and 30 PI with a mean of 8 PI and 14 PI for SAT-1 and SAT-2 sera respectively. Field sera collected from cattle pre-vaccination, post vaccination and sequential sera collected post infection were tested and results compared to those obtained from antibody detection using the LPBE. Although the LPBE was somewhat more sensitive, the SPCE had a higher specificity. This makes the latter test suitable for large scale serological surveys and epidemiological studies, where many sera can quickly be screened at a single dilution.

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Workshop : Bovine tuberculosis

Prevance in buffalo - similar to cattle using PCR. No spillover to KNP employees (1999).

- Which aspects of society are affected by BTB
- Which disciplies are needed.

Group 4 : Community based.

- how do communities want preventative measures communcated? Methods and approaches.