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THE HISTORY OF RIFT VALLEY FEVER IN SOUTH AFRICA

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INTRODUCTION

Rift Valley Fever was first identified in 1931 in the Rift Valley of East Africa. It is a vector borne *Phlebovirus* spread mostly by mosquitoes. This disease causes abortions and deaths in young sheep, cattle and goats. Rift Valley Fever is also a zoonosis and causes a mild flu-like disease in humans, although it can have serious complications such as ocular disease, encephalitis, haemorrhagic disease and even death in a small proportion of affected humans. Humans become infected by coming into contact with blood of infected animals. It is therefore mostly an occupational disease (Swanepoel & Coetzer (2004)).

This disease is associated with above normal rainfall events and is carried over from one rainy season to the next by transovarial transmission of the virus in *Aedes* spp. mosquitoes. These mosquitoes lay their eggs on the grasses surrounding pans/dambos and the eggs must dry out before they can hatch again. Flooding of these pans/dambos causes large numbers of these mosquitoes to hatch, resulting in the infection of livestock. Other mosquito species (e.g. *Culex* spp.) and biting insects can then amplify and spread the disease (Swanepoel (2009)).

Rift Valley Fever in South Africa occurs in sporadic outbreaks or epidemics with long periods, sometimes more than 20 years, of absence. Large parts of South Africa have favourable conditions and the vectors for the disease are present. It is not known if the disease circulates at a low level during inter epidemic periods or if it is re-introduced from the North before each outbreak (Swanepoel & Coetzer (2004)). Not all high rainfall events in South Africa cause Rift Valley Fever outbreaks in South Africa, even after it was known to be present during the previous rainy season. (Directorate of Veterinary Services (2009))

MATERIALS AND METHODS

This paper is the first part of a study to investigate the epidemiology of Rift Valley Fever in South Africa investigating which factors (climatological and other) cause outbreaks in South Africa. The annual reports of the Directorate of Animal Health/Veterinary Services of the National Department of Agriculture of South Africa, disease reports, Provincial Annual reports and the literature referring to Rift Valley Fever were investigated and the place and date of the outbreaks were recorded.

During the second part of this study (in progress) the recorded Rift Valley Fever outbreaks will be compared to other factors like past outbreaks of Rift Valley Fever, rainfall, temperature and outbreaks in neighbouring countries on a temporal and spatial basis.

RESULTS

The results for outbreaks will be given according to the affected rainy season, i.e. from July of one year to June of the following year.

1950-51

Rift Valley Fever was first recorded in South Africa in 1950-51 in the Northern Cape, Western Free State and the then Southern Transvaal. It started in the Western Free State in December 1950 and it continued until April 1951. Specific areas mentioned are the Wolwespruit and Dealesville areas of the Boshof District, Vaalhartz, the Vaal River Barrage, Loskop dam, Koffiefontein and Standerton. The disease was at first not recognised, but after the veterinarian performing a post mortem inspection at the Johannesburg abattoir became sick, it was recognised as Rift Valley Fever. During this outbreak it was recognised that the outbreak was associated with the panveld area (Alexander (1951)).

1951-52

On 30 January 1952 Rift Valley Fever was diagnosed (laboratory confirmed) only on one farm. The farm Weltevreden in the Wolvenspruit area of the Boshof District (Van der Linde (1953)).

1952-53

During April 1953 an outbreak was confirmed in the Gannapan and Legpan areas of the Fauresmith District North West of Luckhoff in the Free State Province. These pans have a geographical reference of 24°40' East and 29°40' South. Farms affected where Eldorado, Legpan, Bossiespan A & B, Wolvenkraal, Nelsdam, Overskot, Koppiesdam, De Rif, Alpha and Wolvenplaat. During this outbreak it was also noted that the outbreak was associated with the flooding of the nearby pans (Van der Linde (1953)).

1953-54

No outbreaks of Rift Valley Fever where found even though several suspected outbreaks where investigated (Alexander (1955)).

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1954-55

A large number of samples for suspected Rift Valley Fever outbreaks where tested, but no outbreaks were found (Alexander (1955)).

1955-56

Twenty-eight outbreaks were reported from the Free State Province in the Wesselsbron, Odendaalsrus, Soutpan, Dealesville, Boshof, Hoopstad, Ventersburg and Bothaville districts (Alexander (1956)).

1957-65

No outbreaks of Rift Valley Fever where reported (Division of Veterinary Services (1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965)).

1965-66

Two suspected outbreaks in the Free State Province could not be confirmed by the laboratory (Division of Veterinary Services (1966)).

1965-66

Several suspected outbreaks were reported, but none could be confirmed (Division of Veterinary Services (1967)).

1967-69

No outbreaks where reported (Division of Veterinary Services (1968, 1969)).

1969-70

Serious losses occurred due to outbreaks reported from the Standerton, Frankfort, Kroonstad, Koppies, Odendaalsrus, Vryburg and the Lower Umfolozi Districts (Division of Veterinary Services (1970)).

1970-73

No outbreaks were reported (Division of Veterinary Services (1971, 1972, 1973))

1973-74

During this period South Africa's largest Rift Valley Fever outbreak occurred. All areas except the winter rainfall area, Eastern Transvaal and Transvaal reported widespread occurrence of the disease. In Natal the disease only occurred in the Eshowe State Vet Area. The Kimberley, Bloemfontein and Fauresmith areas and the Eastern Cape and Karoo suffered severe losses due to the disease. Heavy rains and large tracts of standing water created the ideal conditions for the Rift Valley Fever vectors. Due to a warm winter, the outbreak continued through the winter, spilling over into the next season (Division of Veterinary Services (1974)).

1974-75

The major outbreak of 1973-74 continued during this season, being most severe in the North Western Cape, Southern Free State and the western parts of Transvaal. Other areas specifically mentioned were the Calvinia area, areas along the Orange River, the Nile River Valley near Potgietersrus and East Griqualand. A few cases were reported from Kwazulu Natal (Division of Veterinary Services (1975)). Areas specifically mentioned as being affected by this outbreak in an unpublished report of the Western Cape Veterinary region (Anonymous 1976) are Steinkopf, Springbok, Brandvlei, Sutherland, Williston, Nieuwoudtville, Vredendal, Lutzville, Calvinia (February 1975), Clanwilliam, Van Rhynsdorp (October 1974), Kenhardt (September 1974), Pella (August 1974), Graafwater (May 1975) Lambertsbay and Boesmanland (May 1975).

1975-76

The outbreak continued, but with fewer losses than the previous seasons. The Kenhardt district reported a serious outbreak towards the end of the season and a few cases were reported from the Pietermaritzburg district. No cases were reported from the Northern and Eastern Transvaal region (Division of Veterinary Services (1976)). An unpublished report of the Western Cape Veterinary region (Anonymous (1976)) mentions outbreaks in the Brandvlei area.

1976-77

A few outbreaks were reported from the Mtunzini and Lower Umfolozi districts of Natal and the Queenstown and Middelburg areas of the Eastern Cape (Division of Veterinary Services (1977)).

1977-78

A few cases were reported from the Free State and the Eastern Cape and Karoo regions (Division of Veterinary Services (1978)).

1979-80

A single outbreak was reported in the Mtunzini district of Natal (Division of Veterinary Services (1980)).

1980-81

Several outbreaks of Rift Valley Fever were reported by private veterinarians from the Highveld region, but nothing was laboratory confirmed (Division of Veterinary Services (1981)).

1981-82

Three outbreaks were reported from the Hlabisa district in Natal, one outbreak was reported from the Eastern Cape and Karoo district and the disease also occurred in the Bethlehem and Kroonstad areas regions (Division of Veterinary Services (1982)). A disease report by the Division of Veterinary Services to Southern African Regional Commission for the Conservation and Utilisation of the Soil (SARCCUS) (1983) reported one Rift Valley Fever outbreak during April 1982 so it is very likely that most of the above mentioned outbreaks occurred during the period July 1981 to December 1981.

1982-83

Small outbreaks of Rift Valley Fever were reported from the Natal region and the Highveld region (Division of Veterinary Services (1983)). A disease report by the Division of Veterinary Services to Southern African Regional Commission for the

Conservation and Utilisation of the Soil (SARCCUS) (1984) reported Rift Valley Fever outbreaks during March, April and June of 1983

1983-84

Small outbreaks of Rift Valley Fever were reported from the Natal region and the Western Cape region (Division of Veterinary Services (1984)). A disease report by the Division of Veterinary Services to Southern African Regional Commission for the Conservation and Utilisation of the Soil (SARCCUS) (1984) reported Rift Valley Fever outbreaks during October, November and December of 1983. The report by the Division of Veterinary Services to SARCCUS for the following year (1985) reported one Rift Valley Fever outbreak during February 1984 and one during April 1984.

1984-85

Small outbreaks of Rift Valley Fever were reported from the Utrecht district of Natal, the Kroonstad and Henneman Districts in the Highveld region and a foetus sent from the Transvaal region tested positive for Rift Valley Fever (Division of Veterinary Services (1985)).

1985-86

Thirteen outbreaks of Rift Valley Fever were reported from the Estcourt, Utrecht and Ubombo Districts of the Natal region (Natal (1986)) and 7 outbreaks from the Port Elisabeth, Middelburg, Beaufort West, Hofmeyer and Graaff- Reinet districts of the Eastern Cape and Karoo region (Eastern Cape and Karoo (1986)) (Directorate of Veterinary Services (1986)). The 1986 annual report to the OIE (Directorate of Veterinary Services (1986)) reports two outbreaks during February 1986

1986-87

No cases of Rift Valley Fever were reported (Directorate of Veterinary Services (1987))

1987-90

Even though the climatic conditions were favourable for the disease and the veterinary services predicted outbreaks, no outbreaks were reported. (Directorate of Veterinary Services (1988, 1989, 1990))

1990-91

During February 1991 the Estcourt district of Natal reported 5 outbreaks of Rift Valley Fever and the Kliprivier district of Natal reported 2 outbreaks (Natal veterinary region (1991)).

1991-1992

No outbreaks were reported during this period (Directorate of Veterinary Services (1992)).

1993-1998

According to the Directorate of Animal Health/Veterinary Services Disease database (1993-1998), no outbreaks of Rift Valley Fever were reported.

1999

During January 1999 Rift Valley Fever was diagnosed in Buffalo at Skukuza in the Kruger National Park (Directorate of Animal Health/Veterinary Services Disease database (1999)).

2000-2007

No outbreaks of Rift Valley Fever were reported during this period (Directorate of Animal Health/Veterinary Services Disease database (2000-2007)).

2008

Between January and June 2008 Rift Valley Fever was reported from the Nkomazi, Mbombela and Dr JS Moroka Local Municipalities of Mpumalanga, the Bela-Bela and Ba-Phalaborwa Local Municipalities of Limpopo, the Nokeng tsa Taemane, Tshwane and Kungwini Local Municipalities of Gauteng and the Potchefstroom, Moretele and Madibeng Local Municipalities of the North West Province (Directorate of Animal Health (2008)).

2009

During February to June 2009 several outbreaks of Rift Valley Fever occurred in the Ingwe, Kwa Sani and uMngeni Local Municipalities of Kwazulu Natal and one outbreak in the Mbombela Local Municipality of Mpumalanga (Directorate of Animal Health (2009)).

During October to November 2009 the Northern Cape Province experienced a localised Rift Valley Fever outbreak around the Orange River in the Kakamas region (Directorate of Animal Health (2009)).

2010

A major outbreak of Rift Valley Fever occurred in the first half of 2010. It started in the Bultfontein and Brandfort areas of the Free State Province and spread to all provinces except Kwazulu Natal. The Free State Province, Northern Cape Province and the Eastern Cape Province were severely affected, with the other provinces experiencing lower numbers of outbreaks. More than 500 outbreaks were reported with over 13000 animals reported to have shown clinical signs and over 8000 animal deaths. More than 200 people were affected with 25 deaths. Interestingly, during this outbreak it was the first time the disease spread to the winter rainfall area of the Western Cape Province. It was also the first time the disease was documented in several wildlife and exotic species.

DISCUSSION

The result of this part of the study shows clearly that South Africa has had more Rift Valley Fever outbreaks in the past than what is currently believed. Of the 60 or so years since South Africa's first Rift Valley Fever outbreak, 22 years with Rift Valley Fever outbreaks were experienced. Most of these outbreaks were small; with only a few farms affected and only 3 very large outbreaks were recorded.

It would appear that all the outbreaks mentioned in the Annual Reports of the South African Veterinary Services were investigated and confirmed in the laboratory, because suspected cases that could not be confirmed are also mentioned in some reports and several of the reports clearly state that Rift Valley Fever was confirmed by the laboratory (Onderstepoort Veterinary Institute).

South Africa has had only 3 very large outbreaks of Rift Valley Fever (1950-51, 1974-76 and 2010). These were most likely the result of animal populations that were not properly vaccinated. Farmers are very reluctant to vaccinate for a disease that they do not see very often, despite the fact that Veterinary Services and veterinarians have been warning farmers for many years to vaccinate. The results from this part of the study clearly indicate the need for a sufficiently vaccinated livestock herd.

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RIFT VALLEY FEVER: CURRENT CONCEPTS AND RECENT FINDINGS

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INTRODUCTION

Rift Valley Fever (RVF) is a vector-born infection of ruminants and is also potentially zoonotic. A disease that is compatible with what we now call RVF was first described in in the Rift Valley in Kenya in 1910 – 1912. In 1930, the virus was first isolated in an outbreak of sheep disease in the same locality. During this outbreak, mosquito transmission was demonstrated, and the first human cases were described. Subsequently the presence of this virus was confirmed in many sub-Saharan African countries, demonstrating that it was not confined to the Rift Valley. To date, with the noted exception of the Middle East (Egypt, Saudi Arabia and Yemen) and Madagascar, this virus has remained confined to Sub-Saharan Africa.
The RVF virus is a member of the *Phlebovirus* Genus of the family *Bunyaviridae.* It is a monotypic virus with no significant antigenic differences detected between isolates and laboratory passaged strains. There are however strain differences in pathogenicity.
The virus is stable through a wide range of temperatures, and in serum it can survive heating to 56° C for 3 hours. The virus is rapidly inactivated by pH's below 6.8 and the virus is also inactivated by lipid solvents and formaldehyde.

EPIDEMIOLOGY

RVF is a peracute or acute disease of ruminants. The disease is most severe in sheep, cattle and goats, but has also been reported in water buffaloes, African buffaloes, certain African antelope and camels. Viremic hosts are the source of infection for endemic and epidemic vectors, and viremia may last up to 7 days. Humans are incidental hosts.

ENVIRONMENTAL FACTORS

In sub- Saharan Africa, wet climatic cycles are a feature of *El Nino* southern oscillations and vector abundance is influenced by wet climatic shifts and are frequently associated with increased RVF activity. In addition certain environmental features such as pans, and dambo's (focal and linear depressions where rainwater accumulates) as well as anthropogenic agricultural activities, such as damming of drainages and "flood irrigation" practices may influence RVF activity.

VECTORS OF RVF

THE ENDEMIC CYCLE

The endemic vectors of RVF are certain "flood-water" breeding *Aedine* mosquitoes. These endemic vectors appear to be the viral maintenance hosts during the inter-outbreak / epidemic periods. Transovarial infection occurs in certain gravid female mosquitoes, and these mosquitoes lay their infected eggs on grasses, sedges and mud at the edge of water pools / dambo's and flood plains.
These eggs are dormant and require a drying out period followed by a re-wetting before they hatch. The time frame for this drying and re-wetting cycle may be weeks, months, years or even decades. A certain percentage of the post re-wetting emergent female's offspring will be infected with RVF virus, and may transmit the infection to ruminant hosts. Thus, the infected eggs of these endemic vectors appear to be the mechanism for perpetuation of the virus. In addition, transovarially infected male offspring may sexually transmit the virus to uninfected female mosquitoes, as part of the endemic maintenance cycle. After the infected eggs have hatched, and the adult infected *Aedine* mosquitoes emerge, they are capable of biologically transmitting the infection to ruminants. Under optimum rainfall conditions, the life cycle of these mosquitoes may be completed in as little as 14 - 21 days. Under less ideal environmental conditions, the eggs of these mosquitoes may enter a dormant state, which may extend almost indefinitely.

THE EPIDEMIC CYCLE

Infected viremic ruminants, in turn, become the source of infection for more *Aedine* mosquitoes, as well as a whole host of other hematophagous insects including many other genera of mosquitoes, as well as midges, phlebotomids, stomoxids, simuliuds and tabanids. The populations of all of these hematophagous insects generally peak towards the end of the wet season, and they become important mechanical transmitters of the virus during the epidemic cycle.

ECOZONE PATTERNS OF VIRUS ACTIVITY

The epidemiology of RVF varies with the regional ecozone, landscape and habitat. In moist forest and wet bushed grasslands, RVF generally has an endemic pattern, with low levels of virus activity each year. In drier bushed grasslands, the pattern is one of clear 3 to 15 year inter-epidemic periods, with rainfall / flood related epidemics. In semi-arid to arid zones, 15 – 40 year cycles of epidemic activity occur, generally starting in flood plain areas, followed by spatial spread. Virus emergence is rarely detected in these arid zones during the inter-epidemic periods.

Extension / dissemination of RVF may occur as a result of translocation of infected vectors by weather systems and air currents, passive transport of infected mosquito eggs in mud on the feet of migrating birds, or more commonly, by translocation of viremic animals. In general, areas with hydrological impoundments and dams, as well as irrigation-based agricultural practices are important receptive areas for establishment of disease.

RECOGNITION OF RVF OUTBREAKS

RVF is usually characterized by a sudden outbreak of disease, following above average rainfall, and resulting in deaths of young ruminants (especially lambs and calves) and abortion of pregnant ewes and cows. These outbreaks are frequently accompanied by reports of febrile disease in humans with low mortality.

PATHOGENESIS

RVF virus causes disseminated intravascular coagulation - possibly due to virus induced endothelial damage. In addition, there is a related profound thrombocytopenia as well as diffuse hepatitis.

CLINICAL SIGNS OF RVF IN LIVESTOCK

In young animals, there is a sudden onset of high fever, followed by prostration, collapse and death.

In adult ruminants, abortion is the most important clinical sign. If no abortion occurs, the virus may cause varying grades teratology in the fetus including hydrancephaly, hydrops amnii and arthrogryphosis, all of which may result in later dystocia. In addition, during the acute phase of the disease, the adult ruminants may show anorexia, dysgalactia, nasal and lachrymal discharges, colic, salivation, regurgitation, lymphadenitis, icterus and hemorrhagic enteritis. Mortality also occasionally occurs in adult animals.

The major necropsy finding is that of a hemorrhagic syndrome, the blood does not clot and there may be free blood in the body cavities. There are petechial hemorrhages in the gastro-intestinal tract and on serous surfaces. The liver is enlarged and yellowish to reddish brown with numerous grey or white necrotic foci. These lesions are most pronounced in aborted fetuses and newborn lambs and calves.

Sheep are the most susceptible species, followed by cattle and goats. In indigenous African wild ruminants, the disease has been confirmed in African buffalo, blesbok, springbok, sable antelope, nyala, eland and waterbuck. Amongst exotic wild ruminants in southern Africa, RVF has been confirmed in fallow deer, llamas and alpacas. Camels are the least susceptible, and may occasionally show abortion. In Africa, indigenous livestock breeds are generally more resistant than European breeds. In outbreaks in newly affected areas, there may be a 20 – 90% morbidity, 40 – 60% mortality in young animals and 2 – 5% mortality in adults.

RVF IN HUMANS

Humans are more commonly infected by contact with tissues of diseased animals, than by mosquito bites. The common mosquito vectors are generally sylvatic, and do not enter dwellings. This disease is definitely an occupational hazard for veterinarians, veterinary technicians, abattoir workers and butchers. After an incubation period of 3 – 6 days, typical influenza like symptoms develop, frequently in combination with photophobia, orbital discomfort and visual disturbances. 80% of infections are mild, but some patients will develop hepatic involvement and thrombocytopenia with hemorrhages. 5% of patients develop serious ocular sequelae, and a mortality rate of 0.5 – 1% generally occurs as a result of diffuse hepatitis,a hemorrhagic syndrome and / or encephalitis. RVF in humans is not directly contagious, and barrier nursing is not required.

LABORATORY CONFIRMATION

There are numerous laboratory tests that can be used to confirm a diagnosis of RVF. The RVF virus can be isolated by mouse inoculation or on tissue culture. A real time PCR is available for detecting viral RNA. Antigen detection tests, such as AGID and fluorescent antibody tests are frequently used. Antibody tests including hemagglutination inhibition test, virus neutralization test and ELISA's for IgM and IgG are available. Histopathology and immuno-histochemistry can be used to diagnose the disease from necropsy specimens.

PREVENTION / CONTROL OF RVF IN LIVESTOCK

Routine inoculation of livestock using modified live attenuated Smithburn strain of virus gives lifelong immunity. The vaccine strain is only partially attenuated, and may cause abortions in pregnant females. It is good policy in endemic areas to vaccinate all weaners annually. Predictive use can be made of rainfall estimates and remote sensing of vegetation indices to strategically vaccinate all susceptibles, but the "lead time" may not be adequate. It may be more effective to base strategic vaccination intervention on *El Nino* southern oceans oscillations events. In these cases, pregnant animals should be vaccinated with an inactivated vaccine, which requires a booster after 4- 6 weeks and is very expensive.

Once an outbreak RVF has been confirmed, it is important to define the infected areas and target populations. Thereafter clinical and serological surveillance should be instituted, in high risk flood zones and adjoining areas. Analyze remote sensing and weather data to estimate potential for spread.

The movement, slaughter and consumption of animals in the outbreak areas should be curtailed, and human awareness campaigns using all available media, should be instituted. Vector control using adult insecticides, larvicides and repellants may be instituted in defined areas.

Vaccination in the face of an outbreak may be instituted but is frequently too late for pregnant animals. Beware of needle propagation of infection; a fresh needle must be used for each animal.

With the advent of global climate change, the spatial spread of many vector born infections into previously uninfected zones, regions, countries and continents has become a real threat. RVF is no exception, and the necessary risk mitigation practices to prevent importation of this virus should be heightened.

A REVIEW OF THE PATHOLOGY AND PATHOGENESIS OF RIFT VALLEY FEVER

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ABSTRACT

Rift Valley fever (RVF) is a zoonotic viral haemorrhagic fever (VHF) caused by a mosquito-borne arbovirus belonging to the *Phlebovirus* genus (family *Bunyaviridae*). In the summer of 2010 an extensive outbreak of RVF affecting livestock and humans occurred in South Africa. This outbreak provided the opportunity to re-examine the pathology of RVF. Immunohistochemistry (IHC) and recent significant advances in the understanding of the pathogenesis of viral haemorrhagic fevers provided a framework for the renewed interpretation of the lesions seen in ruminants infected with RVF virus. The most common gross lesions in ruminants included random multifocal areas of hepatic necrosis disseminated throughout the liver accompanied by widespread haemorrhages; severe lung oedema, mild to moderate hydrothorax, hydropericardium and ascites; and marked enlargement and congestion of particularly the mesenteric and the hepatic lymph nodes. Microscopic lesions in ruminants included varying degrees of random multifocal hepatic necrosis; random foci of necrosis in other organs such as the heart, kidneys, adrenal glands, or the intestines; severe lung oedema and congestion; and depletion of the lymphoid tissues. Viral antigen was demonstrated using IHC in hepatocytes, myocytes, adrenal cells, keratinocytes in the epidermis and the tongue, enterocytes and in trophoblasts in the placenta. RVFV-specific positive labeling was also found in infected mononuclear cells and microvascular endothelial cells in the renal glomeruli, the cortical interstitium, the alveolar septa of the lungs, the thymus, the heart and the lymphoid tissues. Models representing our current understanding of viral heamorrhagic fevers have been proposed. Studies indicate that macropahges and dendritic cells (DCs) residing in epithelia are the early targets of these viruses. Presumably, endocytosed virus replicates in their cytoplasm and is conveyed by these cells to the regional lymph nodes. From there the virus disseminates to the liver and other organs where tissue macrophages and/or DCs and parenchymal cells become secondarily infected. Widespread necrosis follows. Cytokines and chemokines released from virus-infected macrophages and hepatocytes dysregulate the host immune response causing lymphocytoclasia, increased vascular permeability and coagulopathy which ultimately results in co-infections, disseminated intravascular coagulation, hepatorenal failure and multiple organ dysfunction.

INTRODUCTION

Rift Valley fever (RVF) is a zoonotic viral haemorrhagic fever caused by a mosquito-borne arbovirus belonging to the *Phlebovirus* genus (family *Bunyaviridae*).[1] It is responsible for large outbreaks of disease in humans and livestock in sub-Saharan Africa, Egypt, Senegal, Mauritania, Yemen, Saudi-Arabia, Sudan, Kenya, Tanzania and Madagascar.[2] In 2008 and 2009 isolated outbreaks of RVF occurred in cattle, buffalo, sheep and goats in Mpumalanga, North West Province, Gauteng, Limpopo, KwaZulu-Natal, Mpumalanga, and the Northern Cape.[3-6] In the summer of 2010 South Africa had abnormally high and persistent rainfall followed by an extensive outbreak of RVF affecting livestock and humans.[7]

Typically farmers reported the near simultaneous abortion of large numbers of pregnant ruminants regardless of the stage of pregnancy. This was accompanied by dystocia in ewes, the death of up to 100% of newborn lambs, and the deaths of significant numbers of sheep and calves as well as a few adult cattle. Recovered foetuses were generally autolysed. However, in sheep herds where the ewes were still in the early stages of pregnancy, often not a single foetus could be found due to the swift actions of predatory birds and carnivores. In these herds the only clue that an abortion storm had occurred was that a large percentages of the ewes presented with soiling of the perineum, and on occasion remnants of the placenta could be seen hanging from the vulva. The abortion rates in cows were generally much lower. Clinical signs sometimes noted in adult ruminants included anorexia, dysgalactia, nasal and lachrymal discharges, salivation, colic, icterus or haemorrhagic enteritis. Typically, examination of the conjunctiva would reveal mild icterus and in some instances petechial haemorrhages. Human infection with Rift Valley fever virus (RVFV) started occurring shortly after the first cases of RVF in domestic livestock were reported.

The diagnosis of RVF, supported by epidemiological data and clinical signs is made histologically. However, many agents cause lesions that superficially resemble those caused by RVF. These may be poisoning with plants such as *Senecio spp*. or *Lantana camara*, and alga like *Microcystis aeruginosa*, as well as bacterial septicaemias such as colibacillosis, salmonellosis and anthrax. Therefore an unequivocal diagnosis can be made only by laboratory tests. The tests used at the Agricultural Research Council-Onderstepoort Veterinary Institute in the 2010 outbreak were virus isolation and polymerase chain reaction (PCR) using published methods.[8-11] In selected cases viral antigen in ruminants was also demonstrated using immunohistochemistry (IHC) at the Veterinary Faculty, University of Pretoria using a published method.[12]

The pathology of RVF in cattle and sheep was re-examined during the outbreak that occurred in the summer of 2010. Astute observations from private veterinarians and specialist pathologists in more than one hundred naturally occurring cases made it possible to determine the frequency with which the observed lesions occur. This review also has taken advantage of the opportunity IHC offered to demonstrate the virus in tissues of cattle and sheep.

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PATHOLOGY

GROSS LESIONS

The most common gross lesions in ruminants included random multifocal areas of hepatic necrosis disseminated throughout the liver accompanied by widespread haemorrhages; severe lung oedema, mild to moderate hydrothorax, hydropericardium and ascites; and marked enlargement and congestion of particularly the mesenteric and the hepatic lymph nodes.

Hepatic involvement was consistently present in neonatal calves and lambs and was characterized by hepatic enlargement and pale yellow or dark reddish brown discoloration. Lambs often had massive necrosis of the liver. The hepatic lesions in most adult sheep and cattle were very distinctive with obvious discrete foci of haemorrhage and necrosis that were sharply delineated from the adjacent parenchyma giving the liver a mottled appearance. The capsular surface was often irregular due to the collapse of liver lobules. The size of such foci was variable, most often ranging from less than 1 mm to several millimeters. In some animals the liver was more severely affected showing large confluent areas of necrosis. Icterus was only infrequently seen in adult animals and very rarely in neonatal animals.

Haemorrhages were most commonly encountered in the subcutaneous tissues, the omentum, and the endocardium. Subcutaneous haemorrhages varied in size from small ecchymoses of less than 10mm in diameter to large suggilations. They were most commonly found on the neck, flanks and limbs. Haemorrhages were also often present in the wall of the gallbladder and the urinary bladder, on the epicardium, in the thymus, and in the lymph nodes. Occasionally haemorrhages were noted on other serosal surfaces such as the spleen or the pleura. Diapedesed blood was often noted around the circumference of the blood vessels in the limbs or the omentum. One neonatal lamb had haemorrhages in both parotid glands.

Most of the ewes that were examined post mortally were pregnant. The placenta often presented with marked necrosis of the cotyledons accompanied by foci of necrosis and haemorrhage in the intercotyledonary placenta. Other than varying degrees of autolysis, occasional hepatomegaly, mild to moderate transudates in the body cavities, and occasional anasarca, no significant lesions could be discerned in most foetuses.

Earlier descriptions of the pathology of RVF also mention oedema of the wall of the gallbladder, haemorrhages at the base of the gallbladder, haemorrhages on the luminal surface of the abomasum, subcapsular haemorrhages in the spleen or the liver, occasional petechiae in the cortex of the kidneys, sometimes partially digested blood in the abomasum, free blood in the lumen of the intestines, and a blood coagulum in the lumen of the gallbladder or blood tinged bile.[13] Most of the gallbladders examined during the 2010 outbreak were macroscopically normal and oedema of the wall of the gallbladder, blood tinged bile or frank blood in the lumen of the gallbladder was only reported infrequently. Sometimes the folds of the abomasums were oedematous and occasionally transmural hemorrhages were noted in the abomasums of calves. A few reports of blood in the abomasum or the intestines were also received. However, most of the neonatal lambs examined had clotted milk in their abomasums and the intestinal contents of adult cattle were in most cases normal. The other lesions mentioned in earlier reports such as subcapsular haemorrhages in the spleen or the liver or haemorrhages in the cortex of the kidneys were rarely observed. Numerous co-infections such as *Escherichia coli* septicaemia, *Clostridial* sp. enteritis or mastitis, *Haemonchus contortus* paracitaemia or infection with *Coxiella burnetti* or *Anaplasma* sp. were frequently diagnosed. Often a diagnosis of RVF was masked by these co-infections. These co-infections are expected since hemorrhagic viruses cause marked immunosupression.[14] This may also account for the dissimilarity in the gross and histological lesions observed in ruminants.

HISTOLOGICAL LESIONS

The most common microscopic lesions in ruminants were varying degrees of random hepatic necrosis, lung oedema and congestion, and depletion of the cells of the lymphatic system. Other organs such as the heart, kidneys, adrenal glands, or the intestines also contained scattered foci of necrosis. The lymph nodes showed marked oedema and congestion and abundant red blood cells were present within the paratrabecular and medullary sinuses. In addition, the red pulp in the spleen and the sinuses of the lymph nodes often contained abundant cellular fragments and fibrin.

The extent of the liver necrosis and the pattern of necrosis seen in ruminants infected with RVFV varied considerably. Lambs and foetuses infected with RVFV tended to present with massive hepatic necrosis while calves, and adult animals more often presented with random multifocal to coalescing areas of hepatic necrosis. In lambs or foetuses the sinusoidal collagen reticulin framework remains for the most part intact and single cells within the framework die and drop out. In calves and adult animals foci of necrosis expand causing a collapse of the collagen reticulin framework. The expanding areas of necrosis eventually coalesce and abundant cell debris and nuclear fragments can be seen within the centre of the necrotic areas. Necrosis was associated with haemorrhage. The portal triads were generally preserved and in most cases scant non-degenerate neutrophils and macrophages were in attendance. Cytolysis aided by inflammatory cells seemed to be the dominant type of cell death. However, a small number of apoptotic cells were sometimes seen in lesions. Eosinophilic intranuclear inclusions were infrequently found in hepatocytes. These inclusions contain NSs protein.[1] In animals that survived for more than a week regeneration in the form of strings of ductular structures were seen. These would mature into hepatocytes in animals that survive the infection.

Severe lung oedema and congestion with areas of atelectasis were often reported. The alveoli were filled with a smooth eosinophilic material and the capillaries within the alveolar walls were congested. Sometimes the interlobular septa were notably

distended by oedema. Depletion of the cells of the lymphatic system and mild to moderate lymphocytoclasia was consistently noted. Occasionally focal haemorrhages were also noted in the thymus, adrenal cortex, lymph nodes, lung parenchyma or in the lamina propria of the abomasum or the small intestines. Necrosis of the cells in the glomeruli and necrosis of cortical tubular epithelial cells was noted on rare occasions.

Histological examination of the tissues did not reveal any signs of fibrinoid necrosis of blood vessels, perivascular inflammation or thrombosis. Neither could overt endothelial necrosis, or the deposition of fibrin in the sinusoids of the liver or in the renal glomeruli be detected. However, biochemical evidence for disseminated intravascular coagulation (elevated fibrin degradation products and decreased fibrinogen) were reported in RVF-infected rhesus monkeys.[15] Perturbation of endothelial cells might lead to intravascular coagulation which would cause reduced tissue perfusion. This would result in extensive cell injury, widespread haemorrhages and death due to shock. IHC utilizing antibodies against fibrin would be valuable to highlight microscopic fibrin thrombi that were not evident on hematoxylin and eosin (HE) stain and provide further insight in the role of DIC in the mechanism of disease of RVFV. This technique was used to demonstrate fibrin deposits in the organs of horses euthanized because of severe gastrointestinal disorders where the prognosis was considered poor.[16]

CORRELATION OF IMMUNOHISTOCHEMICAL LOCALIZATION OF THE VIRUS AND HISTOLOGICAL LESIONS

The extensive utilization of IHC as a diagnostic tool during the 2010 outbreak provided a valuable opportunity to study the distribution of RVFV in tissues. Localization of the virus could also be correlated with the histological lesions noted in HE stained sections and with current knowledge about the pathogenesis of viral haemorrhagic fevers. This facilitated a better interpretation of the lesions.

Generally the cells most heavily infected with RVFV were hepatocytes. In lambs and foetusses, IHC showed that a multitude of randomly located hepatocytes become infected. HE staining showed that the cells become detached and round, with homogenous eosinophilic cytoplasm and retained cell outlines. IHC revealed that the coagulated cytoplasm of these cells was filled with virus. However, in a number of cases virus was not detected in the hepatocyes and the liver showed early signs of repair. On occasion the liver appeared normal. Instead virus was detected in endothelial cells, necrotic cells and mononuclear inflammatory cells in other organs such as the heart, kidneys and the lungs. In calves and older ruminants necrosis seemed to commence in small groups of infected cells. Similar to lambs and foetusses, HE stained cells were detached from their neighboring cells and shrunken. Initially they also had homogenous eosinophilic cytoplasm, retained cell outlines, their nuclei were absent and IHC revealed that their cytoplasm was filled with virus. Seemingly the infection rapidly expands outward leaving cell debris and nuclear fragments in the centre. IHC showed that the centre of the necrotic foci becomes devoid of virus since no labeling of the debris was observed. The cells at the periphery however retained their cell outlines and contained abundant virus. The intervening hepatocytes, between the necrotic areas, did not show RVFV-specific positive labeling. In fact, depending on the extent of the necrosis at the time of death the intervening hepatocytes could appear normal. More often though they showed varying degrees of cell injury characterized by vacuolization of the cytoplasm or in some instances mild steatosis and bile stasis. This could have been due to terminal ischaemic damage of the uninfected hepatocytes as a result of confluent hepatocyte swelling. The latter would produce compression of the vascular sinusoids and loss of the normal more or less radial array of the parenchyma, causing lobular disarray. The deposition of fibrin within the sinusoids could also contribute to the cell injury noted.

Frequently cells in the lymph nodes, spleen, and gut associated lymphoid tissue (GALT) also stained positive for viral antigen. Cells within the red pulp of the spleen stained positive. Endothelial cells within the blood vessels traversing the capsule and trabeculae also contained virus. The infected endothelial cells appeared to be intact suggesting that RVFV does not cause cytolysis of these cells. However, they sometimes appeared to have detached from the basal lamina of the tunica intima since single cells or strings of positive staining cells were sometimes seen lying free within the lumen of the blood vessels. In contrast, cells within the periarteriolar sheaths or the surrounding splenic follicles containing T- and B-lymphocytes respectively did not stain positive for virus. In lymph nodes scattered positively labelled necrotic cells were detected in the subcapsular, paratrabecular and medullary sinuses as well as the endothelial cells lining blood vessels within the lymph nodes including those within the capsule and the trabeculae. T- and B lymphocytes within the lymphatic nodules in the outer cortex, areas within the deep and inner cortex and the medullary cords did not have virus. The necrotic cells observed in the medullary sinuses were most likely macrophages and dendritic cells. Double staining IHC methods using markers for these cells could confirm this assumption. Occasionally necrotic intestinal epithelial cells at the tips of the villi and endothelial cells lining blood vessels, or lymphatic vessels and the GALT within the lamina propria, submucosa and tunica muscularis stained positive for virus.

Frequently swollen microvascular endothelial cells in the renal glomeruli, the cortical interstitium, and the alveolar septa of the lungs showed RVFV-specific positive labeling. On occasion necrotic cells in the renal cortical tubular epithelial cells, and scattered mononuclear cells in the alveoli also had high viral loads. Infected endothelial cells were infrequently also found in the microvascular endothelial cells in the renal medulla or the thymus. Virus was also occasionally detected in the heart where positive staining was generally associated with endothelial cells in the capillaries. However, on rare occasions virus was detected in a few sarcomeres in myocytes. In the adrenal glands, small groups of secretory cells within the zona fasciculata contained abundant virus. On rare occasions skin, tongue or placenta was presented for histological evaluation. Virus was detected in necrotic follicular epithelial cells in the dermis, in keratinocytes in the epidermis, in endothelial cells lining the microvasculature of the dermis, enterocytes or in trophoblasts in the placenta.

PATHOGENESIS

MAJOR VIRULENCE FACTORS OF RIFT VALLEY FEVER VIRUS

Viral haemorrhagic fevers are caused by RNA viruses from four different virus families namely the Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae.[14] The genome of RVFV consists of three RNA genomic segments (L, M, and S).1 The L RNA segment encodes the RNA polymerase. The M genomic segment encodes the structural glycoproteins, G_N and G_C, as well as NSm. The S segment encodes NSs. The glycoproteins, G_N and G_C, contain the only source of neutralizing epitopes. It is very likely that the antibody used for IHC binds to these proteins. The NSs protein is a major virulence factor that forms ribbon-like filaments in the nucleus of RVFV infected host cells.[1] One of its major functions is to counteract the antiviral interferon system.[17-20] NSs suppresses not only the nuclear induction of interferon and therefore gene transcription, but also cytoplasmic transcription of interferon mRNAs. NSs binds to various proteins in the nucleus forming a repressor complex that suppresses transcription of interferon genes. This occurs as early as 3-4 hours after infection. Interferon acts by binding to protein kinase receptors (PKR) on infected host cells thereby activating the enzyme. Once activated PKR mediates a stop in the translation of viral RNA's. However, NSs induces the degradation of PKR by the host cells own protein degradation organelles, called proteosomes, thereby further suppressing this important aspect of the innate immune defense against the virus. NSs protein also causes a drastic reduction in the transcriptional activity of cells infected by RVFV.[21] It does this by sequestering a subunit (p44) of an important basal transcription factor (TFIIH). This event occurs 8 hours after infection. NSs protein also binds heterochromatin clusters of pericentromeric gamma satellite sequences.[22] Targeting of these sequences was correlated with the induction of chromosome cohesion and segregation defects. It is postulated that this could account for the high rate of abortions and teratogenic disorders predominantly observed after RVFV infection with neurotrophic RVFV strains in ovines.

The NSm protein of RVF virus suppresses apoptosis of target cells.[27] Induction of apoptosis in virus infection is one of the host defense mechanisms eliminating infected cells to limit viral replication and spread. Without NSm protein RVFV is not able to amplify efficiently in an infected host since RVFV causes a rapid progression to cell death in virtually all vertebrate cell types infected and requires prolongation of the time of viral replication to secure efficient production of progeny virus. Most humans and animals do not develop fatal RVF disease, presumably due to variations in the innate immune response of the host and exposure of the infected cells to some extracellular factors, such as tumor necrosis factor alpha, that may induce rapid apoptosis prior to the completion of maximum progeny virus production in virus infected cells. This may also prevent massive necrosis of cells in some species or age groups preventing the death of the host and promoting the completion of the life cycle of the virus. Variations in the innate immune response may also be partly responsible for the dissimilarity in the symptoms and the gross and histological lesions observed in different species and age groups.

MECHANISM OF DISEASE OF VIRAL HEMORRHAGIC FEVERS

Most of the viral heamorrhagic fever viruses such as Rift Valley fever, Crimean-Congo hemorrhagic fever (CCHF), Dengue haemorrhagic fever (DHF) and Ebola haemorrhagic fever (EHV) are characterized by fever, hypotension, thromocytopenia, hepatocellular necrosis, occasional bleeding, lymphopenia and lymphoid depletion.[14] Detailed models representing our current understanding of VHF have been proposed.[14,24-25] In addition, studies in nonhuman primates and rodents experimentally infected with Ebola virus, CCHV virus and Dengue haemorrhagic fever virus suggest that some of the antigen presenting cells (macropahges and dendritic cells in particular) are the early targets of these viruses.[26,27] Antigen presenting cells (APCs) are specialized to capture microbial antigens, break them into small peptides, and display these to the appropriate T lymphocytes thereby inciting the adaptive immune response. APCs include macrophages, perisinusoidal macrophages (Kupffer cells) of the liver, Langerhans' cells in the epidermis, and dendritic cells of the spleen and lymph nodes, B lymphocytes, and type II and type III epithelioreticular cells of the thymus.[28] Foremost among these cells are dendritic cells (DCs). Immature DCs reside in epithelia, where they are strategically located to capture entering microbes. Mature DCs are also present in the interstitium of many nonlymphoid organs, such as the heart and lungs, where they can capture microbes that have invaded the tissues. The theory is that early in the infection the virus is endocytosed by macropahges and DCs. Virus replicates in the cytoplasm and is conveyed via lymphatic capillaries to the regional lymph nodes and to the parenchymal cells in the liver, adrenal cortex and other organs. Presumably tissue macrophages and/or DCs and parenchymal cells become secondarily infected and necrosis follows. The specific mechanism of necrosis are not known but might include disruption of metabolic pathways that ensure normal cell architecture and membrane function. In some cases of VHFs fulminant hepatic failure follows. However, hepatocellular lesions are often not significant enough to cause death, and virions are released from infected parenchymal cells into the circulation. Rapid viral dissemination would be aided by suppression of type I interferon response. Following dissemination into the blood vasculature the virus infects microvascular endothelial cells. However, virus does not cause cytolysis of endothelial cells.[29] Instead cytokines and chemokines released from virus-infected macrophages dysregulate the host immune response causing increased vascular permeability and coagulopathy. The coagulation abnormalities vary in nature and magnitude among and within the viral haemorrhagic fevers. For example, Ebola virus induces the overexpression of tissue factor, which results in activation of the clotting pathway and the formation of fibrin in the vasculature.[30] DIC has also been diagnosed in patients with some other VHFs, including CCHF, RVF and DHF. In contrast, coagulation disorders are less marked in Lassa fever, and impairment of endothelial

function contributes to oedema, which seems to be a more prominent finding in Lassa fever than in other VHFs.[31] Supernatants from DCs infected with CCHF virus activate endothelial cells by up-regulating ICAM-1 expression.[26] The function of ICAM-1 is to arrest leucocytes on endothelial surfaces allowing them to migrate into the tissues. However, ICAM-1 up-regulation is not dependent on viral infection. Instead virally induced soluble mediators from DCs activate endothelial cells. The hemodynamic and coagulation disorders common among all of the VHFs are exacerbated by infection of hepatocytes and adrenal cortical cells.[14] Infection of hepatocytes impairs synthesis of important clotting factors. At the same time, reduced synthesis of albumin by hepatocytes results in a reduced plasma osmotic pressure and contributes to oedema. Impaired secretion of steroid synthesizing enzymes by haemorrhagic fever virus infected adrenal cortical cells leads to hypotension and sodium loss with hypovolemia. Hepatorenal syndrome may also play a role in the pathogenesis VHFs with a fatal outcome. This syndrome is occasionally a serious complication of fulminant liver disease. The hallmark of this syndrome is severe renal vasoconstriction. The pathogenesis is not fully understood but probably involves a interplay between disturbances in systemic hemodynamics, activation of vasoconstrictor systems, and a reduction in activity of the vasodilator systems.[32]

Interestingly none of these viruses infects lymphocytes. However, their rapid loss by apoptosis is a prominent feature of these diseases and lymphocytoclasia is often noted.[14] The loss of lymphocytes is likely to result from a combination of factors. For example loss of support from virus infected DCs may result in massive apoptosis. T lymphocytes require stimulatory signals by the interaction of membrane molecules on T lymphocytes and molecules on the APCs to become fully activated and to subsequently differentiate and proliferate. Without these stimulatory signals T lymphocytes undergo apoptosis. Additionally, infected macrophages may upregulate the synthesis of proapoptotic proteins such as nitric oxide, TNF-α, TNF-related apoptosis-inducing ligand. These mediators would also contribute to the deletion of lymphocytes.

CONCLUSION

Many similarities between the pathological lesions and the distribution of virus observed in the 2010 outbreak of RVF and other viral haemorrhagic fevers can be seen from this preliminary study of the available material. The normal distribution of DCs in epithelia and the interstitium of many nonlymphoid organs, such as the heart and lungs would explain the pattern of RVFV-specific positive labeling seen.

Another interesting observation made was that the centre of the necrotic focus becomes devoid of virus since no labeling of these debris was observed using IHC. In addition, the necrotic foci contain nuclear debris. However, it seems like the nuclei of RVFV infected hepatocytes are lost early in the infection and the origin of the nuclear fragments is not clear. Presumably, the nuclear fragments originate from macrophages that had undergone apoptosis. Two types of cell death are described in viral hepatitis namely cytolysis and apoptosis.[33] Cytolysis aided by inflammatory cells seemed to be the dominant type of cell death in RVFV infection. In many viral infections scavenger macrophages would mark sites of hepatocyte loss. However, in the case of RVFV infection, instead of non-degenerated macrophages, abundant nuclear fragments are present. Recent studies suggest that macrophages and dendritic cells are the early targets of viral haemorrhagic fever viruses. [26,27] A possible explanation is that monocytes are recruited to areas of rapidly expanding necrosis but that the virus causes their early demise via apoptosis before any of the debris can be removed.

Recent studies concerning the pathogenesis of viral heamorrhagic fever viruses also indicate that hepatocellular lesions with widespread haemorrhages are not significant enough to explain death. Instead it would seem that disruption of the biochemical pathways in endothelial cells lead to DIC and multiple organ dysfunction. A combination of hepatorenal syndrome, terminal pulmonary oedema, cardiac arrhythmia and co-infections may all contribute to death and account for the variation in the lesions noted.

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AN ATYPICAL OUTBREAK OF RIFT VALLEY FEVER IN THE NORTHERN CAPE IN OCTOBER 2009

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ABSTRACT

In October 2009, an atypical, localized outbreak of Rift Valley Fever occurred in the Kakamas area of the Siyanda District Municipality in the Northern Cape. The Northern Cape Veterinary Services investigated the outbreak looking at three factors, namely: The pre-existence or introduction of the virus in the area; the presence of large populations of susceptible ruminants as well as reports of any mortalities or abortions from the surrounding farms with associated flu-like illness in humans and finally climatic or environmental conditions that encourage a massive build-up in vector mosquito population.

The Rift Valley Fever outbreak in Kakamas was not following heavy and persistent rainfall and the animal population numbers were not sufficient to sustain an epidemic. Two people with a history of contact with infected animals contracted the disease and were confirmed positive. Records indicates that the affected area was part of previous outbreaks notably the 1974/76 outbreak.

INTRODUCTION

Rift Valley fever (RVF) is an arthropod-borne viral disease of ruminants, camels and humans caused by a phlebovirus of family Bunyaviridae. It is also a significant zoonosis which may be encountered as an uncomplicated influenza-like illness, but may also present as a haemorrhagic disease with liver involvement; there may also be ocular or neurological lesions. In animals, RVF may be inapparent in non-pregnant adults, but outbreaks are characterised by the onset of abortions and high neonatal mortality. Jaundice hepatitis and death are seen in older animals.

Outbreaks of RVF are associated with persistent heavy rainfall with sustained flooding and the appearance of large numbers of mosquitoes, the main vector. Localised heavy rainfall is seldom sufficient to create conditions for an outbreak; the simultaneous emergence of large numbers of first generation transovarially infected mosquitoes is also required. After virus amplification in vertebrates, mosquitoes act as secondary vectors to sustain the epidemic.

MATERIALS AND METHODS

According to literature, for epidemics to occur, three factors must be present:

1. The pre-existence or introduction of the virus in the area
2. The presence of large populations of susceptible ruminants;
3. Climatic or environmental conditions that encourage a massive build-up in vector mosquito population.

The latter usually occurs when there are warm conditions and unusually heavy and persistent rainfalls that cause surface flooding and lead to the hatching of infected *Aedes* spp. mosquito eggs and large numbers of vector mosquitoes.

Alternatively, it may occur in the absence of rainfall, where there is a great deal of surface water, as in a river floodplain, originating from heavy rainfall in river basins that may be hundreds of kilometres away in the mountains, or from irrigation (as was the case in the Gezira area of the Sudan and in Egypt).

The above factors formed the basis of our investigation and an outbreak investigation team comprising members of Veterinary Services and Department of Health's Communicable Disease unit carried out the investigation. An area of 3 km around the index farm and along the Orange River was delineated for immediate veterinary attention and the officials of the Department of Health identified Private Doctors, the local hospital and clinics to be visited for rapid alert, health information and giving proper case definition of a suspected and confirmed Rift Valley Fever case in humans.

A simple questionnaire was drawn up which was used by the field team in gathering more information. This questionnaire included human health aspects, land use patterns, rainfall patterns of the last 6 months, census, movement into or out of the area and vaccination history.

In pursuit of point 1, old records of the Department of Agriculture, records of the local museum as well as the local newspaper were also reviewed to check if there are old photos, newspaper clippings or reports of this area having been part of the 1974/75 epidemic.

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RESULTS

The Kakamas area is mainly a grape and wine producing area with few farmers keeping livestock as a side business. The density therefore of livestock in the area is very low. The area is next to the Orange River and has in the past been sprayed for midges (miggies) and mosquitoes by the Department of Agriculture but this had stopped.

Review of some presentations and records of National Department of Agriculture indicates that in "1976, 1 outbreak in the Northern part of the Cape Province near Upington and a few outbreaks in ... (all localized)" were reported. The report talks about "towards the end of the year under review (1976) serious outbreaks occurred in the Kenhardt district".

Table 1: Numbers of animals in the 17 immediate properties (around the index case) that were physically visited.

Cattle	Sheep	Goats
787	1627	30

Field observations did not suggest an over-abundance of mosquitoes in the area although this information was contradictory in the end.

There has not been animal movement into the farm in the last 3 months preceding deaths and abortions in cattle suggesting that it is unlikely that a viraemic animal could have been introduced onto the farm (assuming 8 days of viraemia in cattle).

The reports from the South African Weather Services in terms of rainfall, temperature and wind direction were reviewed and this outbreak was not following heavy rainfall and it is therefore atypical.

The suspicion was then cast on fluctuations of the Orange River which would allow flooding of the surrounding areas creating ideal situation for mosquitoes. The data from the Department of Water Affairs were reviewed and nothing out of the ordinary was observed.

The Boegoeberg dam in Globershoop underwent a routine cleaning operation in July 2009 where a lot of mud / sand was removed. This is the only exercise that was carried out (some suspect that exposing deep sand may expose eggs that have been dormant).

Changing land use is another major factor in the emergence of disease, and in the case of RVF this is reflected in the building of dams or big irrigation areas. No new dams were recently built or are being built in the immediate surroundings or in the Northern Cape along the Orange or Vaal rivers (As was seen in Egypt in 1977 and 1987 in West Africa). There is also no new (virgin) or recent extensive irrigation land being established in the area that could have contributed to the increase of the vector population (as in the 1973 outbreak in Sudan).

DISCUSSION

This outbreak did not conform to what student study material had exposed us to and far more complex factors were at play. The investigation could not conclude what the trigger factors were. From the National Insitute of Communicable Diseases (NICD), we learnt that the virus isolated from humans, was related to the Caprivi virus of 2004. The virus from animals was sent for sequencing but results are still outstanding.
The hypothesis is that since most farmers in the affected area use flood irrigation instead of drip or spray irrigation (cost) and make use of imported raw manure, this may result in ideal conditions for an outbreak. The numbers of ruminants may not have been sufficient to sustain an epidemic.

Further research is needed on the role of manure, trigger factors and vectors initiating the outbreaks. Farmers were given information but uptake of alerts is still a major problem. The role of strategic institutions for technical support should be better defined.

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ONDERSTEPOORT RIFT VALLEY FEVER VIRUS VACCINES

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ABSTRACT

The 2009/10 Rift Valley Fever (RVF) virus disease outbreaks in South Africa have had a significant economic impact on cattle and sheep production. Several cases of RVF in humans, as well as mortalities have also been reported. The erratic nature and failure to detect the epizootic outbreaks at an early stage has made it difficult to control the disease. This has been exacerbated by the generally low degree of resistance or immunity to infection in sheep and cattle herds caused by a poor vaccination programme. Effective and safe vaccines are available for prophylactic purposes, however, vaccination is not compulsory. Onderstepoort Biological Products manufacture a live attenuated vaccine (Smithburn strain) and an inactivated vaccine which are both effective in preventing and controlling the disease when used as part of an immunization schedule. Evaluation of an alternative live virus vaccine, designated as RVF C13 has been investigated for safety and efficacy in sheep, and vaccine field trials in cattle and sheep are continuing. Studies have confirmed the loss of virulence and indicate the suitability of RVF C13 to be used as a safe, cost-effective and efficacious vaccine for the control of RVF. An overview on current vaccines and supporting data for the RVF C13 vaccine will be presented.

INTRODUCTION

Rift Valley Fever (RVF) virus is a single-stranded (-) sense RNA virus of the *Phlebovirus* genus and a member of the *Bunyaviridae* family. RVF is an acute or peracute mosquito-borne disease affecting young, susceptible domestic livestock and game[18]. It is a serious zoonotic disease to veterinarians and workers of the livestock industry who come into direct contact with infectious fluids. The disease is not only an important threat to human and animal health in affected countries, but also impacts negatively on the livelihoods of stock owners, livestock production and the regional and international trade. Although notifiable by OIE international trade codes, control of RVF by means of prophylactic vaccination is not compulsory[14].

The disease is generally non-specific and the first warning is a positive diagnosis of abortions from affected pregnant livestock. Simultaneous occurrence of abortion storms and disease or death in adult ruminants, together with disease in affected humans is characteristic of a RVF outbreak. Conditions precipitating RVF virus disease outbreaks are heavy and prolonged rainfall, floods and unseasonal rains which result in an amplified mosquito population. Transmission to humans is through direct contact with body fluids of infected animals[18]. Human infection from mosquito bites has been documented, but no human to human transmission of RVF has been demonstrated[21].

RVF was first identified in the Rift Valley region of Kenya in1930 and diagnosed as early as 1950 in a severe outbreak in South Africa (SA) and neighbouring countries[18]. Consecutive major outbreaks were recorded in 1974-75 and smaller localised outbreaks in 1981, 1999, 2008-09 and more recently a major outbreak during the first quarter of 2010. Although endemic to the sub-Saharan region and Egypt, the disease has, since 2000, spread to the Middle East, East Africa and Madagascar[5 9 15]. In SA, the 2008 outbreak of RVF affecting cattle, buffalo, and goats was confirmed in 4 provinces - Mpumalanga, North West, Gauteng, and Limpopo. In 2009-10 outbreaks in SA affected thousands of animals in the Free State, Northern Cape, Eastern Cape, and North West provinces[7 13]. Abnormally high rainfall and mild climatic temperature conditions preceded these RVF outbreaks and failure to detect the epizootic outbreaks at an early stage has made it difficult to control the disease. This has been exacerbated by the generally low degree of resistance or immunity to infection in sheep and cattle herds, caused by a poor vaccination programme for several consecutive years.

The only effective control of RVF in livestock relies on prophylactic vaccination. In SA, periodic epizootics of RVF affecting large numbers of susceptible livestock can be correlated with a lack of sustained vaccination (Fig. 1). This results in a sudden demand and often under supply of vaccines in an outbreak, in contrast to the intermittent dry weather periods, when vaccine stock is often destroyed due to product expiry and over production. Between 1952 and 1973, only 10 million doses were issued, compared with 18 million doses during the seven month period of November 1974 to May 1975 during the disease outbreak[1]. RVF vaccine doses sold per year in South Africa for 2002 to 2010 are presented in Fig. 1 [OBP sales figures]. National livestock numbers for 2009 as supplied by the Directorate of Agricultural Statistics, Department of Agriculture, Forestry and Fisheries (DAFF), were estimated at 14 and 25 million for cattle and sheep respectively.

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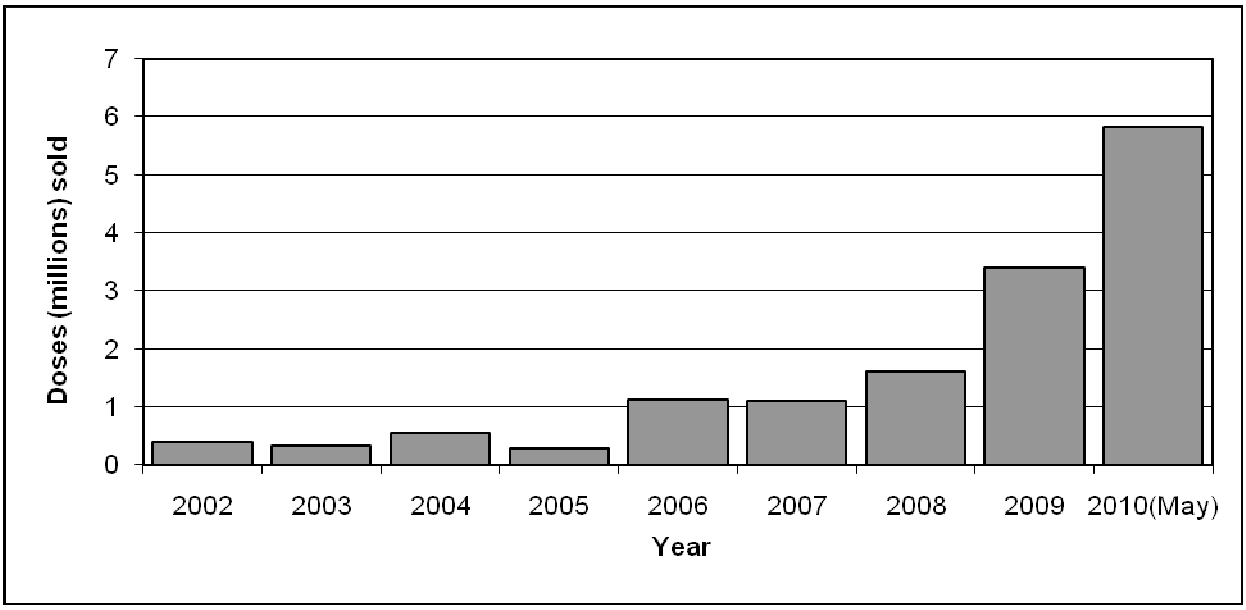

Figure 1: Onderstepoort RVF vaccine doses (live and inactivated) sold in South Africa between 2002 and 2010 (up to May).

Two types of vaccines are currently available from Onderstepoort Biological Products Ltd. (OBP) for use in sheep, goats and cattle: the live attenuated (Smithburn strain) and an inactivated adjuvanted vaccine (virulent field strain). The advantages and disadvantages are described below.

LIVE ATTENUATED RVF SMITHBURN VACCINE

The original Smithburn virus, isolated from mosquitoes collected in the Semliki forest in Western Uganda[17], was attenuated through mouse brain and embryonated hen's eggs at Onderstepoort before being used as vaccine to control RVF in South Africa during 1953-58[1]. The master seed used for production of the OBP Smithburn vaccine since 1971 was further adapted to Baby Hamster Kidney (BHK21) cells[1]. Attenuation of the Smithburn vaccine in mouse brain changed its preference in pathogenicity from pantropism (hepatropism) to neutrotropism and was shown to be safe and immunogenic in vaccinated sheep[16].

Advantages of the attenuated live virus (ALV) vaccine include safety, ease and cost effective production process, relatively short lead time, immunogenicity after a single dose with a long lasting immunity in vaccinated animals. Regular or annual vaccination however, is recommended in endemic areas during spring and before the breeding season. It is advisable, even during inter-epidemic periods, to vaccinate livestock on an annual basis and offspring from vaccinated ewes at six months of age. Newborns of susceptible (unimmunized) dams can be immunized at any age [OBP vaccine product insert][18 20]. Concerns about the safety of the ALV vaccine should be considered when vaccinating animals in their first trimester of pregnancy as the vaccine virus still has an affinity for embryonic tissue, in particular for brain tissue. Vaccination during this period may cause *hydrops amnii* and to a lesser degree teratology of the foetus and prolonged gestation in the dam[6 18]. Over the years the live vaccine has proven to be safe when used in mid- and late-stage pregnant animals.

Use of the live RVF Smithburn vaccine is generally not recommend during disease outbreaks due to the simultaneous presence of the vaccine and wild type virus which may result in an overburden of the infectious agent on the immune system and risk of reversion to virulence, particularly with viruses with segmented genomes. Reversion to virulence is theoretically possible although it was not proven in the case of RVF through experimental host passage and mosquito infection studies[18].

Efficacy studies conducted by the R&D Virology Department, OBP, in young calves have proven that the Onderstepoort RVF Smithburn vaccine is highly immunogenic and protects calves from a virulent RVF virus challenge (data submitted for publication).

INACTIVATED RVF VIRUS VACCINE

The inactivated RVF vaccine is an adjuvanted vaccine which was developed after the 1970's RVF outbreak due to apparent efficacy problems in cattle with the RVF Smithburn vaccine.[1 2] A bovine isolate from an outbreak in the Free State region in 1974 was adapted to BHK 21 cells and used for vaccine production. Due to its virulence it requires production under special conditions in an isolated production facility. This poses an occupational risk in manufacturing and lack of a human vaccine makes continuous production of this vaccine problematic. Production is labour intensive and the down stream process adds to a long lead time and

includes inactivation (formaldehyde) and formulation with an adjuvant (Aluminium hydroxide). All vaccines are tested according to strict quality control standards, which take up to 8 weeks before the vaccine is approved for release into the market.

One major advantage of the inactivated vaccine is its safety for use in pregnant animals and during an outbreak. The disadvantage is that the efficacy of this vaccine relies on a booster vaccination and annual revaccination to maintain an adequate level of protective immunity.

Additional points to be considered on vaccine handling and administration to ensure vaccine efficacy include the following: only use the vaccine as recommended on the vaccine insert; the cold chain (storage at 4°C) must be maintained for both the live and inactivated vaccines; do not freeze the inactivated adjuvanted vaccine; use a new needle for each animal to avoid the possibility of spreading the disease from animals incubating the virus to healthy animals in a herd and discard needles and syringes appropriately after use.

Problems in reaction of animals to vaccine may include: interference from maternal antibodies due to vaccination of young animals born from vaccinated dams at too young an age, the general age and health status of the animals, biological/genetic variation between individual animals, stress and immunodeficiency as well as, the correct diagnosis of the disease causing pathogen.

NEW GENERATION RVF VACCINE

Some of the new generation vaccines that are currently under development include the live attenuated MP12 vaccine, the avirulent natural mutant Clone 13 (C13), an avirulent (laboratory generated) reassortant R566, and various recombinant RVF vaccines as reviewed by Ikegani and Makino[11]. OBP has focused on the development and evaluation of RVF C13 candidate vaccine and a dossier has been submitted for registration to the Registrar (Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 Act 36 of 1984). The original RVF C13 virus was isolated from a non-fatal human case in the Central African Republic. After several passages in mice and Vero cell culture, the virus was plaque purified and tested for homogeneity. A clone designated as 13 was found to be attenuated, non-virulent and highly immunogenic. The lack of virulence was related to a 549 nucleotide deletion (70%) in the NSs gene which is encoded by the S segment of RVF virus genome[12 19]. The RVF virus ss RNA genome is composed of three segments where the large (L) segment codes for the RNA-dependant RNA polymerase, the medium (M) segment codes for the envelope glycoprotein G1 and G2 and two non-structural proteins and the small (S) segment codes for two proteins, the nucleoprotein N and the nonstructural protein NSs[10], the latter which acts as a virulence factor inhibiting host cell protein synthesis and effectively blocks interferon induction during RVF virus infection[3 4].

Advantages of the RVF C13 vaccine are that it is effective in livestock as it has been shown to induce a good protective immunity in sheep and cattle and is safe in pregnant animals. Standard freeze-dried live vaccine production processes are followed which are safe, cost-effective and easy. In addition, the deletion mutant makes an ideal vaccine to distinguish between vaccinated and infected animals in non-endemic areas through the use of assays for differentiation of infected from vaccinated animals (DIVA).

RVF C13 VACCINE: SAFETY AND EFFICACY TESTING IN SHEEP

The safety and efficacy of RVF C13 vaccine in sheep was reported in a paper published by Dungu *et al.*[8]. The study design is presented in Table 1. In short, vaccine safety was evaluated by vaccinating early (within 15 and 50 days of gestation) and late (about 100 days) pregnant ewes, using different vaccine doses (10^4, 10^5 and 10^6 MLD_{50} or PFU per ml). Vaccine safety was further evaluated by testing for viraemia and monitoring body temperatures daily after vaccination and challenge. Protection of the foetuses of vaccinated ewes was evaluated by virulent RVF virus challenge during mid and late pregnancy and monitoring of the development of clinical signs, foetal abortion and development of the newborn lamb. Serum samples were analysed to determine seroconversion following vaccination and challenge. Each experiment included unvaccinated infected control animals (Table 1).

Vaccine safety was demonstrated post-vaccination and post-challenge in that body temperatures did not exceed rectal temperatures of 39°C whereas control ewes were pyrexic. No viraemia could be detected in ewes receiving vaccine doses of $\geq 10^5$ MLD_{50}/ml by cell culture isolation while RVF virus could be isolated in unvaccinated inoculated control sheep. Protection was afforded to all ewes vaccinated with a single vaccine dose of $\geq 10^5$ MLD_{50} or $\geq 10^5$ PFU/ml whereas a low dose of 10^4 PFU/ml failed to protect 1/5 vaccinated ewes challenged with a high virus titre in early pregnancy.

A single vaccine dose induced very good neutralising antibody responses from day 7 which protected ewes during a virulent challenge, preventing abortions and teratogenicity of the unborn lambs. Transfer of maternal antibodies was demonstrated in 20 day old lambs born from vaccinated non-challenged ewes. A dose response effect was evident post-vaccination where high vaccine titres resulted in high antibody responses as presented in Fig. 2.

Table 1: Vaccination – challenge study designs for three independent RVF Clone 13 vaccine experiments using different doses to determine the safety of the vaccine and protection upon challenge with virulent RVF virus (*Dungu et al*., 2010).

	Vaccination			Challenge		
	No. of animals	**Vaccine dose**	**Stage of pregnancy when vaccinated**	**No. Of animals**	**No. of days at challenge**	**Challenge dose**
Exp. 1	Ewes (6)	10^6 MLD_{50}	100 days	3	155 days pv	10^6MLD_{50}, im
	Ewes (7)	10^6 MLD_{50}	50 days	1	120 days pv	10^6MLD_{50}, im
	Rams (2)	10^5 MLD_{50}	-	2	120 days pv	10^6MLD_{50}, im
	Rams (2)	10^4 MLD_{50}	-	2	120 days pv	10^6MLD_{50}, im
	Control ewes (2)	-	-	2	-	10^6MLD_{50}, im
Exp. 2	Ewes (9)	10^5 MLD_{50}	50 days	9	50 pv	10^6MLD_{50}, im
	Control ewes (5)	-	-	5	100 days of pregnancy	10^6MLD_{50}, im
Exp. 3	Ewes (5)	10^4 PFU	15 days	3	30-50 days pv	2 x 10^6 PFU, sc
			15 days	2	90-110 days pv	2 x 10^6 PFU, iv
	Ewes (5)	10^5 PFU	15 days	3	30-50 days pv	2 x 10^6 PFU, sc
			15 days	2	90-110 days pv	2 x 10^6 PFU, iv
	Ewes (6)	10^6 PFU	15 days	3	30-50 days pv	2 x 10^6 PFU, sc
			15 days	3	90-110 days pv	2 x 10^6 PFU, iv
	Control ewes (3)	-	-	2	30-50 days pv	2 x 10^6 PFU, sc
			-	1	90-110 days pv	2 x 10^6 PFU, iv

MLD – mouse lethal dose; PFU – plaque forming units; pv – post-vaccination; im – intramuscular; iv – intraveneous; sc – subcutaneous

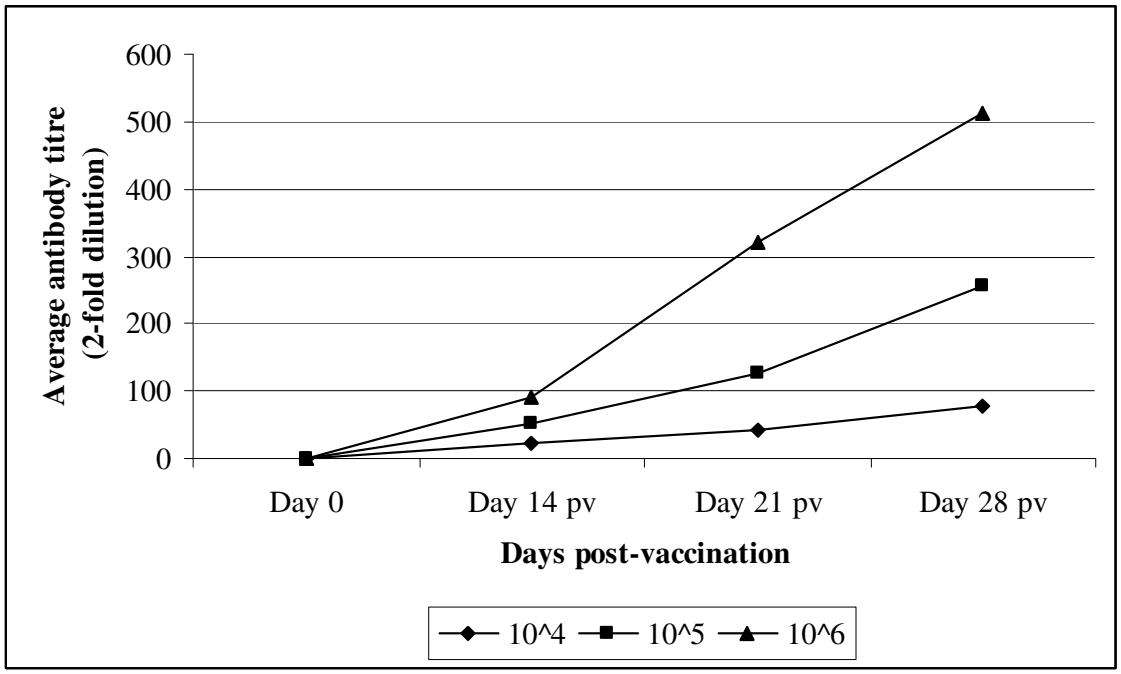

Figure 2: Average neutralising antibody titres of sheep vaccinated with different doses (104, 105 and 106 PFU) of RVF C13 vaccine (Dungu *et al*., 2010).

RVF C13 VACCINE: SAFETY AND EFFICACY TESTING IN CALVES

The safety and efficacy of RVF C13 in calves were subsequently evaluated. Five young RVF-naive mixed-breed calves of 7 months of age were vaccinated subcutaneously with a recommended 2 ml dose (10^5 PFU) of the RVF C13 vaccine. Vaccinated calves were observed for any adverse reactions at the injection site and monitored for febrile reactions by recording of rectal temperatures for 14 days post-vaccination. Vaccinated calves were challenged with 1 ml of virulent RVF virus at 28 days post-vaccination. In addition, five unvaccinated control calves were each inoculated with virulent RVF virus to determine the potency

and to validate the challenge. Animals were observed for the development of clinical signs typical of RVF and rectal temperatures were recorded once daily.

Average body temperatures of calves post-vaccination ranged between 38.6-39.0˚C and a maximum average temperature reading of 38.8°C was recorded on day 2 post-challenge for calves vaccinated with RVF C13. Peak readings in unvaccinated inoculated calves averaged 41°C. Virus titres of 10^6 PFU/ml were consistently recorded in blood from control calves on days 1 to 4 after receiving a high dose of virulent RVF virus. No viraemia could be demonstrated in vaccinated calves post-vaccination or post-challenge. Early and strong neutralising antibody responses of 1:64 and higher were measured in serum collected on day 14 from vaccinated animals and increased to >1:512 following challenge which remained high until the end of the study (Day 63).

All vaccinated calves were protected and remained healthy during the course of the vaccination-challenge trial. Control calves developed acute and subacute clinical signs and were euthanized. These animals showed serum neutralizing antibody responses of less than 1:32 on day 7. Clinical symptoms recorded in control calves with severe acute disease were high fever, diarrhoea, ocular and/or nasal discharge and collapse. Blindness in two unvaccinated, control calves was recorded after virulent RVF challenge (study data in press).

SEROLOGICAL ANALYSES OF CATTLE VACCINATED IN LARGE-SCALE RVF C13 VACCINE FIELD TRIALS

Three herds of cattle (one Afrikaner and two Simmentaler herds) of approximately 1000 head in total were vaccinated with experimental RVF C13 batches to demonstrate vaccine performance in the field. Herds included breeding heifers which were at various stages of pregnancy. Serum samples of about 8% of the vaccinated animals were analysed post-vaccination. A good antibody response was observed by day 14, and antibody titres of naive cattle increased over the first 28 days. Antibodies in cattle that had RVF-specific antibodies at the time of vaccination showed an increase in titres after vaccination. No complications were observed in these herds post-vaccination and cattle were protected in areas were RVF outbreaks were recorded subsequent to vaccination.

CONCLUSION

RVF outbreaks occur once every 2 to 5 decades and can have an enormous impact on the livestock industry and spill over into the human population. The only way to control this vector-borne disease is by ensuring that the livestock population has adequate herd immunity which is achieved through consistent annual vaccination programmes. Ensuring herd immunity during inter-epizootic periods prevents sudden epidemics and/or eliminates endemic RVF virus infections.

Onderstepoort Biological Products Ltd. is the registration holder and manufacturer of two types of RVF vaccine, the attenuated, live Smithburn vaccine and an inactivated vaccine. Both the Smithburn and inactivated vaccines have advantages and disadvantages and must be used as recommended by the manufacturer to ensure vaccine safety and efficacy in recipients. The Smithburn vaccine induces long-term immunity to withstand challenge, however, annual booster vaccinations are recommended during inter-epidemic periods of RVF disease as immunity declines in the absence of antigenic stimulus. Vaccinating animals with the Smithburn vaccine during early stages of pregnancy has a potential risk to cause teratogenicity, while the inactivated RVF vaccine is safe at any stage of pregnancy. The inactivated vaccine is effective provided a booster vaccination is administered within 3-4 weeks of the initial vaccination to ensure protection.

A new candidate RVF C13 vaccine is currently awaiting registration with Act 36 of 1947. The production process of RVF C13 vaccine is cost- and time effective. The vaccine is safe for use in sheep and cattle irrespective of the pregnancy status, protects against virulent RVF challenge and has proven effective during field vaccine studies in target animals. RVF C13 allows for DIVA vaccination which, together with accompanied diagnostic assays may become critical as a trading tool.

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MOLECULAR TYPING OF *CLOSTRIDIUM PERFRINGENS* TYPES AMONG ANIMALS IN SOUTH AFRICA

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SUMMARY

Of the microorganisms that cause human and veterinary illnesses, *Clostridium perfringens* is deemed as one of the commonly encountered pathogens. These bacteria cause enterotoxaemia that may be characterised by enteritis, sudden death and diarrhoea. The toxins of *C. perfringens* determine their virulence and they are classified as types A, B, C, D and E depending on whether they express the lethal alpha, beta, epsilon or iota toxins. In neonatal animals, transmission of *C. perfringens* toxin is through the dam. Therefore, it is important to detect *C. perfringens* types in a specific geographical area in order to develop relevant vaccines. As the information regarding *C. perfringens* types in South Africa is limited, an indication of the predominant *C. perfringens* types can be obtained from submissions that were made at the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI). The aim of this retrospective study was to determine the predominant types of *C. perfringens* strains among animals in South Africa based on submissions from January 2009 to November 2009. Anaerobically grown *C. perfringens* isolates were obtained from various animal species. These isolates were characterized by PCR that targeted alpha, beta, epsilon and iota genes. The amplicons were electrophoresed through 1.5% ethidium-bromide stained gels, followed by observation under ultraviolet light. Reference strains of the *C. perfringens* types A, B, C and D were included as controls for each preparation. The PCR revealed that 51 of the 58 (87.9%) *C. perfringens* that were identified during the 10 month period were *type* A, and 7 isolates (12.1%) were type D. Although *C. perfringens* type A forms part of the normal intestinal flora of animals, the enterotoxin producers have been linked to enteritis in animals and food poisoning outbreaks in humans. The high frequency of *C. perfringens* type A among different animal species in general supports their veterinary and public health importance despite the low numbers of submissions during the study period. Inclusion of the *C. perfringens* types obtained in this study in existing vaccines may enhance their effectiveness.

KEY WORDS

C. perfringens, PCR, toxin genes, animals

INTRODUCTION

Clostridium perfringens are the aetiological agents of a diverse range of diseases in humans and animals, including clostridial enterotoxaemias, which are usually characterized by acute and fatal intoxications (Al-khaldi, Villanueva, & Chizhikov, V (2004)). These spore-forming Gram positive non-motile bacterial pathogens elaborate enterotoxins that are responsible for the pathogenicity of the organism in sheep, piglets, and calves (Quinn, Carter, Markey & Carter (1994)). The main toxins that are produced by *C. perfringens* are alpha, beta, epsilon and iota. Indeed, these toxins are linked to the virulence and *C. perfringens* type, which depends on the various toxin combinations that are elaborated by the bacteria. Based on these major lethal toxins, *C. perfringens* is divided into types A, B, C, D and E, which all commonly produce the alpha toxin (Effat (2008)).

Type A are autochthonous to human and animal intestinal tracts and most soils and the alpha toxin is the predominant product (Yoo, Lee, Park & Park. (1997)). Despite being native inhabitants of animal and human intestinal tracts, *C. perfringens* type A may be associated with myonencrosis and haemolysis (Daube, China, Simon, Hvala & Mainil (1994)). *Clostridium perfringens* type B produces alpha, beta and epsilon toxins and has been associated with enterotoxaemia in sheep and goats. Type C of *C. perfringens* produces alpha and beta toxins. Beta toxin is crucial for pathogenesis of necrotic enteritis in animals and man (Quinn *et al*. (1994)). Type D of *C. perfringens* produces epsilon and alpha toxins. Type E of *C. perfringens* produces alpha and iota toxins.

Characterization of *C. perfringens* toxin types using colonial morphology, biochemical properties, fatty acid analyses and gas-liquid chromatography is extremely challenging. Therefore, *C. perfringens* toxin types have been characterised using seroneutralization in mice or guinea pigs (Daube *et al*. (1994)). Even so, this method is not only time consuming, but numerous antitoxin antibodies and animals are required (Kalender, Ertas, Cetinkaya, Muz, Arslan & Kilic (2005)). Alternatively, a more

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rapid and accurate PCR method was used for typing *C. perfringens* (Yoo *et al*. (1997); Baums, Schotte, Amtsberg & Goethe (2004)).

In South Africa, there is paucity of information regarding *C. perfringens* types. However, an indication of the predominant *C. perfringens* types may be obtained from analyses of submissions that were made to the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI). Therefore, the aim of this retrospective study was to determine the predominant types of *C. perfringens* strains among various animals species in South Africa based on submissions from January 2009 to November 2009. Such information is important in order to develop relevant vaccines that confer specific immunity for a particular geographical zone.

MATERIALS AND METHODS

PHENOTYPIC ANALYSES

Samples from various organs such as intestine, liver, lung and spleen were collected from infected animals and submitted to the General Bacteriology laboratory of the ARC-OVI for analyses. The predominant *C. perfringens* types were investigated among South African bovine (19), caprine (6), ostriches (6), porcine (6), ovine (19), canines (1), avian (4), sitatunga/ marshbuck (*Tragelaphus spekii*) (1) and sable antelope (1) species. The samples were inoculated directly onto 5% sheep blood-tryptose agar (BTA), followed by anaerobic incubation at 37°C for 48 h. The shape, size and texture of the bacterial colonies were examined. The 58 isolates that showed a double-zone of haemolysis, which is characteristic of *C. perfringens* were purified and subjected to Gram staining and microscopy, catalase, litmus milk, thiogel and lecithinase tests.

All Gram positive isolates were subjected to biochemical characterization whereby sugar fermentation patterns were observed after inoculation of glucose and lactose, followed by anaerobic incubation at 37°C for 48 h. The sugars were then examined for the production of gas and acid and the results were interpreted as described by Quinn *et al*. (1994)).

MOLECULAR ANALYSES

PREPARATION OF DNA

All the isolates that were assigned to *C. perfringens* by biochemical tests were subjected to molecular analyses. For this purpose, DNA was extracted by cell-lysis using the rapid boiling method as described by Theron *et al*. (2000) with slight modifications. Briefly, presumptive overnight cultures of *C. perfringens* isolates were scraped from BTA and suspended in 1 ml molecular grade water contained in eppendorf tubes, followed by boiling of the suspension for 20 min. The cell debri was removed by centrifugation at 13 000 rpm for 5 min. The supernatants were transferred to sterile 1.5 ml eppendorf tubes and subsequently used as template DNA in the PCR reactions.

PCR AMPLIFICATION OF TOXIN GENES

Amplification of alpha, beta and epsilon toxin genes was carried out using three sets of primers that were described by Yoo *et al.* (1997). The specific primers for each toxin gene are listed in Table 1. The 25 µl reaction contained 3 µl of template DNA (bacterial lysate), 2 µl of 10 mM dNTPs, 2.5 µl of 10 X PCR buffer, 1 µl of each of the primers (20 µM; Inqaba Biotechnologies, Pretoria, South Africa), 1.5 µl of $MgCl_2$ (25mM), 0.2 µl of Supertherm *Taq* polymerase (5U/ µl; Southern Cross, Cape Town, South Africa) and 13.8 µl of molecular grade water. Reference strains of the *C. perfringens* types A, B, C and D were included as positive controls for each preparation. *Escherichia coli* DNA and molecular grade water were included as negative controls. The PCR mixtures were placed in an Eppendorf thermocycler (New York, USA) and subjected to the following thermocycling conditions: predenaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min. To ensure extension of all products, a final extension step at 72°C for 5 min was performed after the last cycle. The PCR amplicons (15 µl) were analysed by electrophoresis on a 1.5% (w/v) agarose gel stained with ethidium bromide (5µg/ ml), followed by visualization under ultraviolet light. A 100 bp DNA ladder was included in all the gels as size marker (Inqaba Biotechnologies).

RESULTS

PHENOTYPING

Out of the 63 submissions of known animal species that were made to the ARC-OVI for analysis over the 10 month period (January 2009 to November 2009), 58 isolates exhibited the attributes of *C. perfringens* as described in Quinn *et al*. (1994). The colonies were smooth, grey, convex and showed a double-zone of haemolysis on BTA. Microscopical examination of the presumptive *C. perfringens* showed Gram positive rods. The presumptive *C. perfringens* were catalase and lecithinase positive and they hydrolysed gelatin. These bacteria produced gas and acid from litmus milk, glucose and lactose.

GENOTYPING

The PCR results (Fig. 1) revealed that 51 of the 58 (87.9%) *C. perfringens* that were identified during the 10 month period were *type* A, and 7 isolates (12.1%) were type D. *Clostridium perfringens* type A revealed a 402 bp amplicon that is consistent with the alpha toxin gene (Effat, Abdallah, Soheir & Rady (2007)). The 402 bp band was found in all the *C. pefringens* isolates among all the species in this study. Type D of *C. perfringens* showed two bands that represented the 402 bp alpha and 541 bp epsilon toxin genes. Type D of *C. perfringens* was found among caprine (2 isolates), bovine (1 isolate) and ovine (4 isolates).

DISCUSSION

The mode of infection of *C. perfringens* is linked to the toxins that are elaborated by these bacteria. Indeed, the combinations of the major toxins determine the *C. perfringens* type. In this retrospective study, 58 of the 63 submissions of known animal species that were sent to ARC-OVI over a 10 month period were assigned to *C. perfringens* using morphological and biochemical tests. The double-zone of haemolysis caused by *C. perfringens* on BTA is due to theta (haemolysin) toxins, which may play a part in tissue damage (Quinn *et al*. (1994)). Molecular characterization using PCR amplification revealed that 51 of the 58 (87.9%) *C. perfringens* were *type* A, whilst 7 of these isolates (12.1%) were type D. Both these toxin types of *C. perfringens* have been linked to diseases in animals and humans.

The predominance of *C. perfringens* type A among various animal species in South Africa is significant as pathogenic strains of these bacteria are responsible for causing enterotoxaemia in animals (Quinn *et al*. (1994)). Pathogenic type A of *C. perfringens* usually harbour a medically important toxin named CPE, hence from a molecular epidemiology view point, it is paramount to distinguish those isolates that possesses the CPE gene (Miyamoto, Wen & McClane (2004)). The predominance of type A of *C. perfringens* among various animal species in South Africa is consistent with the findings of other researchers (Das, Dutta, Devriese & Phykan (1997); Effat *et al*. (2007)).

Although the prevalence of *C. perfringens* type D was relatively low, the presence of these bacteria is important as they are responsible for causing pulpy kidney (Quinn *et al*. (1994)). In addition, Type D of *C. perfringens* poses serious economic constraints for livestock producers due to the elaboration of epsilon toxin, which induces cerebral edema and brain tissue necrosis (Yoo *et al*. (1997)). The type D of *C. perfringens* was isolated in 4 ovine, 2 caprine and 1 bovine. This is in harmony with the notion that type D of *C. perfringens* is rare in calves. In conclusion, the PCR technique that was used in this retrospective study was rapid and specific; hence it continues to be important for typing *C. perfringens* among animals in South Africa. Types A and D of *C. perfringens* are predominant among various animal species in South Africa and may be important for inclusion in existing vaccines to enhance their effectiveness.

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Table 1: Summary of primer sequences used in this study*

Primer	Primer sequence	Amplicon size
Alpha toxin (cpa)	Forward: 5'-gttgatagcgcaggacatgttaag-3' Reverse: 5'-catgtagtcatctgttccagcatc-3'	402 bp
Beta toxin (cpb)	Forward: 5'-actatacagacagatcattcaacc-3' Reverse: 5'-ttaggagcagttagaactacagac-3'	236 bp
Epsilon toxin (cpe)	Forward: 5'-actgcaactactactcatactgtg-3' Reverse: 5'-ctggtgccttaatagaaagactcc-3'	541 bp

***All primer sequences were taken from Yoo *et al*. (1997).**

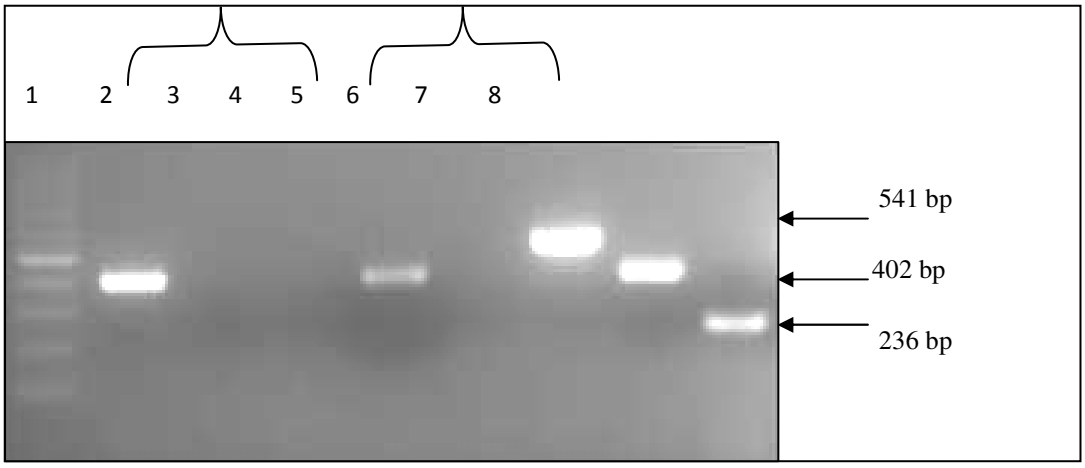

Figure 1: A typical PCR profile showing amplified toxin genes. Lane 1: 100 bp DNA size marker; lanes 2, 3 and 4: one field isolate that is positive for alpha toxin gene only; lanes 5, 6 and 7: one field isolate that is positive for alpha and epsilon toxin genes representing type D of *C. perfringens*; lanes 8 and 9: *C. perfringens* reference strains showing alpha and beta-toxin genes respectively.

AVIAN INFLUENZA IN SOUTH AFRICA: A REVIEW

C. Abolnik[1,2]

SUMMARY

All sixteen subtypes of influenza A viruses occur in their low pathogenic form in wild aquatic birds, in a symbiosis that neither causes clinical disease in the host nor drives these RNA viruses of the Orthomyxoviridae family to mutate. When the viruses are transmitted to susceptible poultry, mild disease may occur. The real threat however, not only to poultry but also to humans and other mammals, are the genetically unstable H5 and H7 subtypes, that mutate to highly pathogenic forms, spread quickly, cause severe disease and are expensive to contain and eradicate, with added trade penalties. In this paper I recap what we have learned about the prevalence of influenza A viruses in local wild birds in the region, through various surveillance programmes aimed at identifying viruses by molecular methods, and how these viruses have been involved in outbreaks in local poultry utilising molecular and phylogenetic comparisons of the strains that have been isolated from ostriches, chickens, ducks and wild birds since 2004. Wild birds remain important sentinels and early warning systems for emerging strains of influenza A viruses that potentially threaten the poultry, animal and human populations of the region.

INTRODUCTION

Avian influenza has been around for decades and possibly even centuries, but it made a spectacular leap into the global spotlight in 2006 when HPAI H5N1 arose in wild birds in East Asia and eventually spread westwards into Europe and Africa, affecting not only birds but various mammalian species including humans, with an alarming fatality rate of 59% in the latter case (WHO, www.who.int (2010); Vandegrift *et al*. (2010)). More recently, H1N1 influenza ("swine flu") virus, a triple reassortment of human, swine and avian influenza viruses has made headlines, for much the same reason that HPAI H5N1 did: fears that these strains or their progeny could be involved in the next human pandemic, which would likely kill millions of immunologically-naïve people, if history is any indicator (Vandegrift *et al*., 2010).

Avian influenza is caused by influenza A virus (AIV), an orthomyxovirus (negative-sense single-stranded RNA viruses) with a segmented genome encoding ten known proteins: hemagluttinin H and neuramindase N (the two major surface antigens), as well as the "internal" proteins matrix M1 and M2, non-structural proteins NS1 and NS2, nucleoprotein NP, polymerase basic protein PB2, polymerase basic protein PB1, and polymerase A PA) (Neumann *et al*., 2003). The implications of an RNA genome is that the virus is prone to rapid mutations due to lack of proof-reading functions, and since the genome is segmented, they are prone to reassortment (shuffling) when two viruses happen to co-infect a cell. Combined, these two attributes produce a pathogen that is capable of adapting to its host and spreading rather quickly, although host range restrictions do apply. Wild ducks are the recognized natural reservoirs of all 16 H and 9 N types, and infection is usually sub-clinical. The virus replicates in the respiratory and gastro-intestinal tract and is spread by aerosol droplets or faeces of infected birds (Webster *et al*., 1978; Röhm *et al*., 1996).

For regulatory purposes, two main types of avian influenza are defined: (1) notifiable influenza (NAI), caused by the H5 and H7 subtypes, that may fall into either the low pathogenic avian influenza (LPNAI) (causing mild symptoms and low mortalities in poultry) or highly pathogenic avian influenza (HPNAI) (severe disease with high mortalities in poultry), and (2) non-notifiable influenza, or LPAI, consisting of the remaining 14 H subtypes (H1-H4; H6; H8-H16) (also usually only cause mild disease in poultry). HPAI H5 and H7 do not constitute separate genetic lineages; rather they arise by mutation in terrestrial poultry, presumably via a mechanism where the terrestrial host polymerase "stutters" in replicating a specific portion of RNA in the hemaggluttinin gene, viz. the HA_0 cleavage site that forms the major virulence determinant. The OIE definition of HPNAI at the molecular level relies on the presence of multiple basic amino acids arginine (R) and lysine (K) at HA_0. Other genomic markers of host adaptation and virulence or attenuation are systematically being identified and characterized. In addition to molecular markers, the intra-venous pathogenicity index (IVPI) test in chickens is required to demonstrate HPNAI (Röhm *et al*. (1995); Alexander (2000); Spackman *et al*. (2003); OIE (2009)).

AVIAN INFLUENZA IN OSTRICHES

AI is traditionally associated with ostriches in South Africa. The Western Cape Province (Klein Karoo region) represents 70-80% of the South African ostrich-farming industry, where the main production systems, export- slaughter and processing facilities are situated, but contract growers in the Eastern Cape and Northern Cape Provinces also raise slaughter ostriches for the larger producers. The production system is unique since it is based on extensive (free-ranging) farming procedures (Olivier (2006)). The Klein Karoo is a semi-arid region with winter rainfall, thus farms tend to be concentrated along rivers and irrigation schemes. Flood-irrigated lucerne

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pastures are planted for younger chicks, with open grazing pastures for slaughter birds. Farmers also produce wheat and barley for ostriches to graze in winter. The aforementioned factors, along with open water systems such as farm dams, create ideal environments that attract various wild bird species, most notably waterfowl. This results in close contact between waterfowl and ostriches, ideal for the transmission of AI (Olivier (2006)).

Despite the existence of extensive farming in the Klein Karoo region since the late 1800's, it was only in the 1980s that ostrich farmers in the Oudtshoorn area first reported a syndrome which they described as "green urine" with respiratory signs. The green urine syndrome appeared to be a seasonal occurrence, peaking in the autumn and winter months (rainy season), but was also associated with periods of drought. Then, in 1991 an LPAI H7N1 virus was isolated during an outbreak in young ostriches under the age of 8 months with symptoms of green urine. Adult birds did not appear to be affected. An IVPI test in chickens indicated that the virus was of low pathogenicity (score of 0.00) and the sequence at HA_0 lacked multiple basic amino acids (Table 1) (Allwright *et al.* (1993)).
Since 1991, and prompted by the seasonal appearance of a combination of certain clinical signs in ostrich flocks (including green urine, respiratory distress, depression, ruffled feathers, diarrhoea, poor appetite, acute hepatitis, peritonitis, respiratory pathology, increased mortalites etc.), various AI viruses have been isolated (Table 1). The aforementioned clinical signs are however not pathognomonic for AI in ostriches (Olivier (2006)).

In 2004 HPAI H5N2 was detected on a feedlot farm in the Eastern Cape Province which resulted in the implementation of control measures and mass destruction of 30 000 infected and in-contact ostriches. The SA Government suspended all export and international trade partners placed a ban on importation of all fresh ostrich products. An intensive epidemiological survey that followed with serological and PCR testing seemed to indicate that HPAI had not spread beyond the Eastern Cape Province; however, 50 of 463 farms tested in the Western Cape were serologically positive for exposure to H5 on hemagglutination inhibition (HI) tests. Since no virus could be detected neither through isolation in eggs nor by PCR, it is assumed that the positive serology results indicated prior circulation of an H5 strain, although neither mortalities nor clinical signs were ever reported from that province (Sinclair *et al.* (2006)).

The export bans were eventually lifted by the European Union in October 2005, but in 2006 HPAI H5N2 was again detected, this time in the Western Cape. Young ostriches (4 months of age) were again involved, but neither significant mortalities nor clinical signs were reported (Abolnik (2007)). The immediate concern was that the 2004 outbreak strain had not been eradicated from ostriches, or had been circulating in an unknown reservoir. Subsequent molecular genomic characterisation at ARC-OVI ruled out the possibility:

1. the 2006 HPAI outbreak strain had fewer basic amino acid insertions compared to HPAI H5N2 from 2004 (Table 1); basic amino acids are usually accumulated through serial passage in the terrestrial host (Spackman *et al.* (2003)) and reversion of loss of basic amino acids at HA_0 has never been recorded.
2. a closer phylogenetic relationship was demonstrated with an LPAI H5N2 virus isolated around the same time in the same province from ostriches than with the 2004 HPAI H5N2 strain
3. multiple reassortments with wild bird AIV genes was demonstrated: the internal gene composition of the 2006 HPAI H5N2 virus was fundamentally different from that of the 2004 HPAI H5N2 strain.

Thus, the two outbreaks were unrelated and represent the second time in less than three years when LPNAI viruses circulating in ostriches mutated into HPNAI. The frequency with which these events occurred seemed remarkable.

Infection with AI and even HPAI in ostriches does not cause serious mortalities (Manvell *et al.* (2003); Oliver (2006)). The increased mortalities in ostriches in the 2004 LPNAI H7N1, 2004 HPNAI H5N2 and 2006 HPNAI H5N2 outbreaks (as well as the occasional outbreaks of LPAI strains such as H6N8) were ascribed to factors such as secondary infections with other pathogens, high population densities, inadequate ventilation and bad hygiene resulting from poor management practices (Allwright *et al.* (1993); Olivier (2006); Abolnik (2009)). HPNAI ostrich AI viruses do not even appear to be initially virulent in chickens, but do gain virulence once passaged in chickens. The 2004 HPAI ostrich strain had an initial IVPI value of 0.63 in chickens, and after further passage in embryonated fowls' eggs an elevated value of 1.19 obtained. Cloacal swabs from the initial IVPI chickens was then taken, passaged in eggs, and after another IVPI a value of 2.73 was recorded. Surviving chickens from the first two IVPIs developed cyanosis of the wattles, combs and legs and became depressed, but remarkably recovered to a normal clinical state after the ten day period (Abolnik *et al.* (2009)). The 2006 HPNAI H5N2 strain similarly produced a low IVPI score (0.58) (Ruth Manvell, personal communication).

AVIAN INFLUENZA IN CHICKENS

Only one of the AIV types, viz. H6N2, has been detected in SA chickens to date. The infection appears to have become endemic, and started in the Camperdown region of KwaZulu-Natal some time around June 2001. Although not notifiable nor the cause of high mortalities in chickens, production losses have been recorded for infected farms. Molecular and phylogenetic characterisation was

used to determine the origins of the outbreak, with interesting results. Firstly, two distinct sub-lineages of H6N2 circulated at the start of the outbreak. One of these contained certain molecular markers for adaptation to chickens (22-amino acid stalk deletion in the neuraminidase protein gene, a predicted increased N-glycosylation, and a D^{144} mutation of the hemagglutinin protein gene) and spread to the Gauteng Province, probably via the movement of eggs between commercial operations or by vendors of spent hens. These data supported other published accounts that H6 viruses are capable of forming stable lineages in chickens (Webby *et al*. (2003); Woolcock *et al*., (2003)). Notably, the two sub-lineages shared a recent common ancestor or progenitor. Secondly, it is most probable that the H6N2 progenitor originated from a reassortment of ostrich viruses or their progeny, viz. the H9N2 strain of 1995 and the H6N8 strain of 1998 (Table 1), with the implication that ostriches acted as a mixing vessel for the chicken outbreak strain. Unsubstantiated reports suggest that the source of the Camperdown outbreak was a consignment of infected chickens from the Western Cape. Sporadic outbreaks continued, and by 2005 H6N2 was being detected in the Western Cape, Gauteng, KwaZulu-Natal and North West Provinces in chickens, ostriches and doves. Thirdly however, these later outbreaks were found to be caused by the progenitor H6N2 strain (Abolnik *et al*. (2007b)). Nevertheless, controlled vaccination for H6N2 with an inactivated virus was allowed, which has in all likelihood further contributed torwards the endemicity of the infection. The true status and impact of H6N2 infection in South Africa remains unknown, since 6-monthly compulsory surveillance is restricted to the detection of NAI (H5 and H7).

AVIAN INFLUENZA IN DOMESTIC DUCKS

Intensive duck farming is not widely practiced in South Africa (due partly to our relatively arid climate) and only one outbreak of AI in domestic ducks (*Anas platyrhynchos domestica*) has been reported thus far. In early February 2009, sudden increases in mortality of 100 to 150 ducks per day were recorded on a commercial duck farm at Joostenburgvlakte in the Western Cape Province. Most deaths occurred in a group of birds with “facial abcesses”. Four weeks later the mortalities had dropped, but samples submitted to the Stellenbosch Provincial Veterinary Laboratory were confirmed positive for *E.coli* and AIV infection. The AI virus that was isolated was identified as LPAI H10N7 by RT-PCR and sequencing. IVPI testing at VLA-Weybridge Laboratory (UK) determined the IVPI score to be 0.00, and the mortalities in ducks were therefore attributed to secondary bacterial infections. When the owner of the farm was questioned, he indicated that he had ceased to use borehole and municipal water due to high costs, and had started pumping in water from a nearby open dam around the time of the first onset of clinical signs. This dam has a resident population of Sacred ibises (*Threskiornis aethiopicus*) and Egyptian geese (*Alopochen aegypticus*) and subsequent testing of fresh faeces by real-time reverse transcription PCR indicated the presence of AIV. These viruses were identified as the H10 subtype by gene-specific conventional RT-PCR, and phylogenetic analysis subsequently confirmed wild birds as the source of infection on the duck farm (Abolnik *et al*. (2010)).

AVIAN INFLUENZA IN WILD BIRDS

The first report of avian influenza in South Africa is internationally recognized as the first report of avian influenza occurring in wild birds. 1300 Common terns (Sterna hirundo) perished along the Western Cape coast between Port Elizabeth and Lamberts Bay in late April 1961. The orthomyxovirus that was isolated from the dead birds was initially named “Tern virus”, and later determined to be an HPAI H5N3 strain (Becker, 1966). The first active surveillance of wild birds for AIV in SA only occurred some 35+ years later, where 262 aquatic birds comprising 14 species captured in the Oudtshoorn region during late autumn and winter of 1998 were tested for AIV (Pfitzer et al. (2000)). The investigators not only demonstrated the presence of antibodies to AIV in the wild ducks, but also isolated several H10N9 strains (Table 1). The next targeted study was undertaken in 2004, at the Baberspan wetlands in southern Gauteng, and H3N8, H4N8 and LPNAI H5N1 was isolated from various endemic anatid species (Table 1). Since then, wild bird surveillance of ducks and shorebirds has continued on an annual basis (data on the prevalence will soon be published elsewhere). The only virus isolated so far, despite occasional PCR detection of the AIV group, was an H1N8 virus from an Egyptian goose (Abolnik et al. (2010)). An interesting case where a North-American lineage partial LPNAI H5 sequence was amplified by RT-PCRR from the H3N8 sample was notable, because it demonstrated cross-continental sharing of influenza virus genes (Abolnik, 2007a). In all other cases, endemic anatid AIV genes have had close evolutionary relationships with Eurasian viruses (that form a separate lineage from North American strains), implying that long-distance migratory birds (i.e. shorebirds) are somehow involved in the periodic introduction of AIVs into the South African environment where endemic vectors become infected and propagate further (Abolnik et al. (2006)).

WILD DUCKS AND AI EPIDEMIOLOGY

There is no doubt that wild ducks play a major role in the epidemiology of AIV in South Africa, as the source of infections in poultry, as repeatedly demonstrated by molecular characterization and phylogenetic analyses. One of the objectives of phylogenetic analysis is to define the gene pool of AI viruses in SA. Genomic segment reassortments give rise to new combinations of genes that indicate the intermingling of duck populations across provincial borders (Abolnik *et al*., (2010)). We are also monitoring for the introduction of genes from HPAI-H5N1 infected regions. The hypothesis that anatids from southern African mix and share viruses with eastern,

western and northern Africa birds can hopefully be supported or discredited as ongoing studies in other regions contribute sequence data to the public databases.

Serological evidence of H5 infection on ostrich farms in the Western Cape in 2004 tended to suggest that the H5N2 outbreak had started in that province, and had circulated undetected prior to being transmitted to the Eastern Cape (Sinclair *et al*. (2006)). Initially it was assumed that transmission could only have occurred through the movements of infected ostriches or contaminated vehicles, equipment or persons. Fortuitously a likely LPAI H5N2 precursor had been detected by RT-PCR and sequencing in a wild Egyptian goose in the Oudtshoon region just prior to the outbreak in Eastern Cape Province (Abolnik *et al*. (2009)). Transmission of HPAI by wild ducks had not been considered a viable method of spread until a paper by Gaidet *et al.* was published in 2009. They detected HPAI H5N2 in an apparently healthy White-faced whistling duck (*Dendrocygna viduata*) and Spur-winged goose (*Plectropterus gambensis*) in a Nigerian wetland. The sequences at HA_0 were determined to be PQKEKRRKKR*GLF and PQREKRRKKR*GLF respectively. The latter amino acid motif was identical to that of the 2004 HPAI H5N2 ostrich strain (Table 1). The Nigerian duck AIV H5 and N2 sequences were phylogenetically closely-related to the South African strains isolated/ detected in both 2004 and 2006, but do not appear to be directly derived from the South African outbreaks. What the study does suggest however is that certain lineages of HPAI H5 are able to asymptomatically infect wild ducks, and the possibility that ducks introduced the HPAI infection from the Western Cape to the Eastern Cape Province in 2004 therefore cannot be ruled out.

PREDICTION AND RISK MAPPING

The ostrich export leather and meat industry is economically important (and an international leader with 60% of the world share market), bringing in 1.2 billion rand annually and supporting around 18 000 direct jobs (SA Ostrich Business Chamber statistics; Olivier (2006)). In comparison, the gross income from chickens (meat and eggs) contributes 30,1516 billion rand annually (24% of all agricultural production) and can literally claim to feed the nation (DAFF statistics, 2009). The economic effects of HPNAI outbreaks through culling, loss of valuable breeding stock and the closing of export markets are devastating to the poultry industry, notwithstanding the potential impact of AIV strains with pathogenic potential on a large HIV-compromised human population. The development of prediction and risk models for AIV has thus been a focus for various groups in recent years. Thompson *et al* (2008) used a questionnaire-based census to identify risk factors associated with H5 seropositive farms in the Western Cape Province. They found that increased risk of farm-level H5 AI seropositivity was associated with increasing numbers of ostriches, reduced cleaning of feed troughs and a failure to clean and disinfect transport vehicles. In the two main regions surveyed, increased risk was associated with increasing frequency of contact of ostriches with wild birds but particularly with white storks (*Ciconia ciconia*) in both regions, with gulls (*Larus* spp.) in the Southern Cape and with Egyptian geese in the Klein Karoo. However, more than 100 white storks and gulls samples from the Western Cape have so far tested negative for the presence of AIV (ARC-OVI unpublished laboratory data). Similarly, more than 600 white storks were sampled and tested in Germany between 2003 and 2008, and none of the nestlings or adults tested positive for AIV infection. Only two storks found dead in April 2006 tested positive for HPNAI H5N1 during the European epidemic (Müller *et al*. (2009). The role of white storks in AIV epidemiology in the ostrich farming areas thus remains doubtful.

Cumming *et al* (2008) attempted to map the risks for avian influenza transmission by wild ducks in southern Africa. They developed risk values for each of 16 southern African anatid species and summed risk estimates, combining these with environmental risks. The areas with the highest risk values were those near the largest two cities, Johannesburg and Cape Town, although parts of KwaZulu-Natal and the Eastern Cape also had high risk scores. Their predictions do not, however, fit with what is historically observed in South Africa, viz. the sporadic infection of ostriches with AIV in the Klein Karoo region. Since backyard chicken flocks most certainly do exist, the reasons why regular transmissions of AIVs from wild ducks do not occur are speculatory, but probably relate to the relatively arid southern African environment. Cumming *et al*. (2008) did however present a valuable assessment of risk evaluation for individual anatid species. They assessed 16 local species based on range, abundance, mobility, roost, mixed flocks, foraging and anthropogenic association. Egyptian geese achieved the highest risk score of 87 across all categories, followed by Red-billed teal (*Anas erythrorhyncha*),81; Spur-winged goose (*Plectropterus gambensis*), 75; White-faced whistling duck (*Dendrocygna viduata*), 74; Yellow-billed duck (*Anas undulata*) ,74; South African Shelduck (*Tadorna cana*), 69; Comb Duck (*Sarkidiornis melanotos*), 69; Cape Shoveller (*Anas smithii*), 68; Cape Teal (*Anas capensis*), 67; Southern Pochard (*Netta erythrophthalma*), 63; Fulvous duck (*Dendrocygna bicolor*), 61; Hottentot Teal (*Anas hottentota*), 56; White-backed duck (*Thalassornis leuconotus*), 50; Maccoa Duck (*Oxyura maccoa*), 48; African Pygmy-Goose (*Nettapus auritus*), 44; and African Black Duck (*Anas sparsa*), 44. When this list is compared with the species from which AI has been isolated or detected in South Africa (Table 1), the correlation is evident. For this reason, Egyptian geese are considered to be the best sentinels for AIV and are the focus of specific intensified surveillance projects currently underway.

CONCLUSIONS

Routine AIV surveillance in wild birds is a relatively recent effort in South Africa, and is labour-intensive. Our veterinary infrastructure and resources are stretched over wide and often remote geographical areas, thus the future for surveillance for AI in South Africa is to work smarter, not harder. This will most likely involve the targeting of sentinel populations or species, and hopefully even artificial or biological sensors placed in the environment that can be monitored remotely. There is an urgent need to generate and overlay accurate spacial maps of poultry density with climatic and environmental data, agricultural data (especially cultivated grain crops favored by wild birds), and wild bird prevalence. More can be done from a regulatory point regulate to monitor how poultry are moved from hatching to rearing to slaughter and processing sites across provincial borders, including the movements of cull buyers. Much more work is to be done in order to fully understand the complex dynamic among AIV, the wild bird reservoir and poultry hosts, and the environment in South Africa.

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Table 1. Incidence of influenza A viruses in South Africa

Year of isolation	Location	Virus	Subtype	Host	HA_0	Comments	Reference
1959; 1961	Lambert's Bay	A/tern/South Africa/1961	H5N3	*Sterna hirundo*	PQRETRRQKR*GLF	HPNAI Isolation also described in 1959?	Becker (1966), USDA AIV sequencing project
1991	Klein Karoo district	A/ostrich/South Africa/1991	H7N1	*Struthio camelus*	PEIPKGR*GLF	LPNAI	Manvell *et al*. (1996)
1994	Klein Karoo district	Lab number/ location unknown	H5N9	*Struthio camelus*	Unknown	Location of viral stocks unknown	Pfitzer *et al*. (2000)
199?	Klein Karoo district	Lab number/ location unknown	H5N2[1]	*Struthio camelus*	unknown	See comment (1) in footnote	Pfitzer *et al*. (2000)
199?	Klein Karoo district	Lab number/ location unknown	H9N5	*Struthio camelus*	unknown		Pfitzer *et al*. (2000)
1995	Klein Karoo district	A/ostrich/South Africa /9508103/95	H9N2	*Struthio camelus*	PAASYR*GLF		Li *et al*., Genbank submission (unpublished?)
1998	Oudtshoorn	Lab number/ location unknown	H10N9	*Alopochen aegyticus, Hadeda ibis, yellow-billed duck, shelduck*	unknown		Pfitzer *et al*. (2000)
1998	Oudtshoorn	N/A	H6N?	*Alopochen aegypticus*		serology	Pfitzer *et al*. (2000)
1998	Leeu Gamka district	A/ostrich/South Africa/KK98/98	H6N8	*Struthio camelus*	PQIEPR*GLR		Abolnik *et al*., (2007b)
2001	Klein Karoo district	A/ostrich/South Africa/2001	H10N1	*Struthio camelus*	Unsequenced	Unsequenced	Olivier
2001-2010	All provinces	A/chicken/South Africa/AL19/02 A/chicken/South Africa /UP1102/02	H6N2	*Gallus gallus, Various*	PQIEPR*GLR/ PQIETR*GLR	Endemic	Abolnik *et al*. (2007b)
2004	Oudtshoorn	A/Egyptian goose/South Africa/AI23/04	H5N2[2]	*Alopochen aegypticus*	PQRETR*GLF	See comment (2) in footnote	Abolnik *et al*. (2009)
2004	Eastern Cape	A/ostrich/South Africa/N227/04	H5N2	*Struthio camelus*	PQREKRRKKR*GLF	HPNAI IVPI=0.63	Abolnik *et al*. (2009)
2004	Blesbokspruit	A/Duck/South Africa/1108/04	H3N8	*Anas capensis/ Anas smithii*	PEKQTR*GLF	Organ pool	Abolnik *et al*. (2006)
2004	Blesbokspruit	A/Duck/South Africa/1233A/04	H4N8	*Anas erythrorhynchos*	PEKASR*GLF		Abolnik *et al*. (2006)
2004	Blesbokspruit	A/Duck/South Africa/811/04	H5N1	*Anas undulata*	PQRETR*GFL	Virus not isolated	Abolnik *et al*. (2006)
2006	Western Cape	A/ostrich/South Africa/AI1160/06	H5N2	*Struthio camelus*	PQRETR*GLF	LPNAI IVPI=0.00	Abolnik (2007)

2006	Albertinia/ Riversdale district	A/ostrich/South Africa/AI1091/06	H5N2	*Struthio camelus*	PQRRKKR*GLF	HPNAI IVPI=0.56	Abolnik (2007)
2007	Oudtshoorn	A/ostrich/South Africa/AI1447/07	H6N8	*Struthio camelus*	PIETR*GLF		Abolnik *et al*. (2010)
2007	Baberspan	A/Egyptian goose/South Africa/AI1448/07	H1N8	*Alopochen aegypticus*	PSIQSR*GLF		Abolnik *et al*. (2010)
2007?	Mosselbaai	AI1472	H5N8	*Sterna bergii*	Not determined	Virus not isolated	Abolnik *et al*. (2010)
2008	Oudtshoorn	A/Egyptian goose/South Africa/AI1556/08	H4N2	*Alopochen aegypticus*	PEKASR*GLF		Abolnik *et al*. (2010)
2008	Oudtshoorn	A/ostrich/South Africa/AI1586/08	H9N2	*Struthio camelus*	PAVSDR*GLF		Abolnik *et al*. (2010)
2009	Joostenburgvlakte	A/Pekin duck/South Africa/AI1642/09	H10N7	*Anas platyrhynchos domestica*	PEIMQGR*GLF	IVPI=0.00	Abolnik *et al*. (2010)

[1]Some literature cites the isolation of an LPAI H5N2 virus from ostriches in Zimbabwe in 1995 and 1996 (Pfitzer *et al*. (2000); Olivier (2006)), however this was later determined to be a laboratory contamination (R Horner, personal communication).

[2]This virus was not "lost" (Olivier (2006), Sinclair *et al*. (2006)). Detection was done directly on RNA extracted by tissues, a partial H and full N sequences were obtained, as well as sequences for the NS and M genes. The material had been freeze-thawed, and was rotting, thus no viable viruses remained for successful isolation in eggs.

REVIEW OF WILD BIRDS AND THEIR ROLE IN THE EPIDEMIOLOGY OF AVIAN INFLUENZA WITH PARTICULAR REFERENCE TO WATERFOWL IN SOUTH AFRICA

D.J.Verwoerd

SUMMARY

Investigations into the role of wild birds, particularly waterfowl, in the epidemiology of both LPAI and HPAI have been intensified in recent years due to the clear association with outbreaks in the Northern hemisphere. Waterfowl can act as primary introducers as well as agents of secondary spread. Traditional perspectives are inadequate, particularly in understanding these dynamics in the Southern African context where local, opportunistic movements by waterfowl, as well as agricultural and socio economic developments influence the functioning of ecological mixing vessels in the form of permanent water bodies and reliable food sources. An ecohealth perspective is particularly appropriate for the understanding and control of avian influenza in this region.

INTRODUCTION

Wild ducks are the main reservoirs of influenza A viruses that can be transmitted to domestic poultry and mammals, including humans. Of the 16 hemagglutinin (HA) subtypes of influenza A viruses, only the H5 and H7 subtypes cause highly pathogenic (HP) influenza in the natural hosts. Several duck species are naturally resistant to HP Asian H5N1 influenza viruses. These duck species can shed and spread virus from both the respiratory and intestinal tracts while showing few or no disease signs. While the HP Asian H5N1 viruses are 100% lethal for chickens and other gallinaceous poultry, the absence of disease signs in some duck species has led to the concept that ducks are the "Trojan horses" of H5N1 in their surreptitious spread of virus (Kim et al 2009).
Recent introductions of highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds and its subsequent spread throughout Asia, the Middle East, Africa and Europe has emphasised the role of wild birds in the geographical spread of HPAI H5N1 virus. Large-scale surveillance programs are ongoing to determine a potential role of wild birds in H5N1 virus spread and to serve as sentinel systems for introductions into new geographical regions. The unprecedented scale and coverage of these surveillance programs offer a unique opportunity to expand our current knowledge on the ecology of LPAI and HPAI in wild migratory birds. (Munster et al 2009) The transmission of viruses and their geographical spread is dependent on the ecology of the migrating hosts. For instance, migrating birds rarely fly the full distance between breeding and non-breeding areas without stopping over and "refueling" along the way. Rather, birds make frequent stopovers during migration and spend more time eating and preparing for migration than actively performing flights. Many species aggregate at favorable stopover or wintering sites, resulting in high local densities. Such sites may be important for transmission of LPAI viruses between wild and domesticated birds and between different species. Prominent, large areas with high potential for mixing of Eurasian and African ducks are in West Africa, near the Senegal and Niger Rivers, the flood-plains of the Niger River in Nigeria and Mali, and Lake Chad, and influenza viruses in African Anatidae populations may thus be linked to Eurasia through migrating species. (*Olsen* et al 2006)

EXAMPLES OF PRIMARY INTRODUCTION BY LONG-DISTANCE MIGRANTS

During the northern hemisphere spring migration of 2005 (April) the largest known mortality in wild birds caused by a HPAI H5N1 strain occurred at Lake Qinghai in NW China (Tibet). Estimations vary between 1500-6000 dead migratory waterfowl of several species, mostly Bar headed Geese. Circumstantial evidence strongly suggested wild birds as the origin of the outbreak although speculations regarding the role of local farming with semi-domesticated Bar headed Geese, or the possibility that human /infected poultry movements brought the virus to the region clouded the issue. Soon after, in August 2005, a similar event occurred at the remote Lake Erkhel in Mongolia, caused by the same strain of HPAI. This was investigated more thoroughly that was still in the region trying to investigate aspects of the Qinghai outbreak. They collected 774 samples from both dead and living birds at Lake Erkhel and the USDA confirmed HPAI H5N1 in dead birds, but none found in the samples from live ducks, geese, swans, gulls. Wild bird movements are the most likely explanation of this outbreak. (Liu et al 2005)
Retrospective analyses of the series of HPAI H5N1 outbreaks during 2005-2006 in Romanian village poultry, could be predicted by the distance from migratory waterfowl sites.(Ward et al 2008)
Several species of dabbling ducks and diving ducks (8 birds from each species challenged, 4 from each species negative [placebo] controls) were experimentally infected with HPAI H5N1, and their health and excretion patterns studied. All birds were captive bred and reared indoors for 8-11months as well as screened negative before challenge. Tufted ducks, Eurasian Pochards and Mallards excreted significantly more virus than Common Teals, Eurasian Wigeons and Gadwalls, yet only the Tufted Ducks and to a lesser degree the Pochards became ill and died. Thus some wild ducks species, especially Mallards can potentially be long distance vectors of HPAI, while others such as Tufted ducks can be utilised as sentinels. (Keawcharoen 2008)

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POSSIBLE MECHANISMS WHEREBY BIRDS CAN REMAIN LONGTERM CARRIERS /SHEDDERS OF HPAI

It has been argued that infected birds would be too severely affected to continue migration and thus unlikely to spread the H5N1 virus. Although this may be true for some wild birds, it has been shown that, in experimental infections, several bird species survive infection and shed the H5N1 virus without apparent disease signs (Sturm-Ramirez (2004), Hulse-Post et al*.,* (2005). In addition, many wild birds may be partially immune owing to previous exposures to LPAI influenza viruses, as has been shown for chickens (Seo et al 2002). Finally, recent studies suggest that HPAI viruses may become less pathogenic to ducks infected experimentally, while retaining high pathogenicity for chickens (Hulse-Post et al (2005), Sturm-Ramirez *et al* (2005).The present situation in Europe, where infected wild birds have been found in several countries that have not reported outbreaks among poultry, suggests that wild birds can indeed carry the virus to previously unaffected areas. Although swan deaths have been the first indicator for the presence of the H5N1 virus in several European countries, this does not necessarily imply a role as predominant vectors; they could merely have functioned as sentinel birds infected via other migrating bird species.
The pathobiology of H5N1 HPAI virus infection in wild waterfowl is poorly understood. A recent study (Kwon et al 2010) examined the pathobiology of A/chicken/Korea/IS/06 (H5N1) HPAI in 5 migratory waterfowl species-mute swans (Cygnus olor), greylag geese (Anser anser), ruddy shelducks (Tadorna ferruginea), mandarin ducks (Aix galericulata), and mallard ducks (Anas platyrhynchos)-following intranasal inoculation or contact exposure, from which all birds became infected. In mute swans, this virus had strong vascular endothelial cell tropism, producing acute severe disease and 100% mortality; the virus was detected in various parenchymal cells; and necrotic and inflammatory changes were noted in a range of organs, including pancreas, brain, spleen, heart, oral cavity, adrenal gland, lung, and liver. The ruddy shelducks had 100% mortality, but time to death was delayed, and the lesions were primarily restricted to the brain, heart, pancreas, and spleen. The mandarin ducks had only a single mortality, with lesions similar to those in ruddy shelducks. The greylag geese became infected, developed neurological signs, and had residual meningoencephalitis when examined at termination but lacked mortality. The mallards had asymptomatic infection. These results indicate variation in the pathobiology of H5N1 virus infections in different species of wild waterfowl, ranging from severe, acute systemic disease with 100% mortality to asymptomatic infection of respiratory and gastrointestinal systems.

EXAMPLE OF SECONDARY SPREAD THROUGH LOCAL MIGRATION

The controversial potential existence of a wild bird reservoir for highly pathogenic avian influenza (HPAI) has been questioned recently by the spread and the persisting circulation of H5N1 HPAI viruses, responsible for concurrent outbreaks in migratory and domestic birds over Asia, Europe, and Africa. During a large-scale surveillance programme over Eastern Europe, the Middle East, and Africa,(Gaidet et al 2008) the investigators detected avian influenza viruses of H5N2 subtype with a highly pathogenic (HP) viral genotype in healthy birds of two wild waterfowl species sampled in Nigeria. They monitored the survival and regional movements of one of the infected birds, a White faced Whistling Duck, through satellite telemetry, providing rare evidence of a non-lethal natural infection by an HP viral genotype in wild birds. Phylogenetic analysis of the H5N2 viruses revealed close genetic relationships with H5 viruses of low pathogenicity circulating in Eurasian wild and domestic ducks. In addition, genetic analysis did not reveal known gallinaceous poultry adaptive mutations, suggesting that the emergence of HP strains could have taken place in either wild or domestic ducks or in non-gallinaceous species. The presence of coexisting but genetically distinguishable avian influenza viruses with an HP viral genotype in two cohabiting species of wild waterfowl, with evidence of non-lethal infection at least in one species and without evidence of prior extensive circulation of the virus in domestic poultry, suggest that some strains with a potential high pathogenicity for poultry could be maintained in a community of wild waterfowl. The White-faced Whistling Duck is a particularly good candidate for this role as it is a resident species widespread over South America and Africa south of the Sahara, including Madagascar and the Comoro islands. Across most of Africa it is a partial intra-African migrant, also showing nomadic tendencies, moving in relation to water levels and food availability, for example in Western Africa between Sahelian and savannah areas. It is the most abundant resident duck in Africa, with numbers estimated at between about 1,000,000 and 2,000,000 birds (with 600,000-700,000 in West Africa)
A large body of scientific literature indicate the consequences of the wild waterfowl contribution to the “mix” of a LPAI pool, mutating to HPAI in poultry: (www.who.int/csr/disease/avian_influenza/en/)

1. Circulating pool of all known LPAI H and N types in wild waterfowl, spillover to poultry (and ostriches); mutation to HPAI. Documented at least 19 times since 1959.

2. 1996/7 - 2005/6 SE Asian H5N1poultry outbreaks…53 countries, 256 human cases, 151 human deaths . Hundreds of millions of chickens, turkeys, ducks, geese died / culled plus trade restrictions = impact of >$10 Billion

EXAMPLE OF LEGAL/ILLEGAL AVICULTURAL TRADE AS MECHANISM OF PRIMARY /SECONDARY INTRODUCTION OF HPAI

On October 18, 2004, 2 Crested Hawk-Eagles (*Spizaetus nipalensis*) smuggled into Europe from Thailand were seized at Brussels International Airport (Van Borm et al 2005). Clinical examination of the birds showed no symptoms. As import of birds and products from several Asian countries into the European Union (EU) is forbidden (DG Sanco Decision 2004/122/EC), the birds were humanely sacrificed and immediately sent to the Veterinary and Agrochemical Research Centre for routine diagnosis to exclude influenza and Newcastle disease viruses, where a HPAI were indeed isolated. This report demonstrates that international air travel and smuggling represent potential threats for introducing and disseminating H5N1 virus worldwide.

PREDICTING AND MODELLING

The spread of highly pathogenic H5N1 avian influenza into Asia, Europe, and Africa has resulted in enormous impacts on the poultry industry and presents an important threat to human health. The pathways by which the virus has and will spread between countries have been debated extensively, but have yet to be analyzed comprehensively and quantitatively. This study (Kilpatrick et al 2006) integrated data on phylogenetic relationships of virus isolates, migratory bird movements, and trade in poultry and wild birds to determine the pathway for 52 individual introduction events into countries and predict future spread. They show that 9 of 21 of H5N1 introductions to countries in Asia were most likely through poultry, and 3 of 21 were most likely through migrating birds. In contrast, spread to most (20/23) countries in Europe was most likely through migratory birds. Spread in Africa was likely partly by poultry (2/8 introductions) and partly by migrating birds (3/8). Their analyses predict that H5N1 is more likely to be introduced into the Western Hemisphere through infected poultry and into the mainland United States by subsequent movement of migrating birds from neighboring countries, rather than from eastern Siberia. These results highlight the potential synergism between trade and wild animal movement in the emergence and pandemic spread of pathogens and demonstrate the value of predictive models for disease control.

Migratory birds mix with domestic / free ranging commercial poultry, ducks and geese by sharing the same surface water bodies. They also indirectly share waterborne disease agents with commercial housed poultry and pigs as well as humans, where basic water sanitation practices are inadequate and surface water are used for drinking purposes. Pigs act as the classic “mixing vessel”, allowing antigenic shift as they are susceptible to both mammalian origin as well as avian origin Influenza A viruses, and thus allowing the reassortment of different strains into completely new ones. The traditional farming practices in South East Asia where rice paddies, domestic waterfowl and poultry, people and pigs all live in close proximity to eachother, create the perfect combination of elements for the development of new HPAI strains with human pandemic potential.

In the northern hemisphere the movements of ducks and other waterfowl are dictated by seasonal and breeding related migratory patterns established over millennia, while exact timing, routes and congregations are the results of weather fronts. In contrast African ducks have evolved generally as nomadic opportunists that move unpredictably based on the short-term availability of food and shelter (Oatley & Prys-Jones 1986). These are determined by widely fluctuating regional weather patterns. Modern agriculture has changed this phenomenon to the extent that artificial dams; both large and small, now provide suitable habitat throughout the year, while extensive grain farming and crop irrigation as well as effluent from intensive pig farms and cattle feedlots in addition to sewage works around towns and cities provide concentrated areas of easily available food. Almost annual “outbreaks” of LPAI and/or HPAI in ostriches in the Little Karoo region of South Africa illustrate this trend.(Pfitzer et al 2000). The nett result is large, localized multispecies populations of waterbirds that comingle with migratory populations of similar / different species. This situation provides ample opportunities for co-infection, exchange of different strains, etc so that these “new” environments should be seen as “ecological mixing vessels”.

ENVIRONMENTAL INFLUENCES ON THE ROLE OF WILD BIRDS IN THE EPIDEMIOLOGY OF AVIAN INFLUENZA

AGRICULTURE

Wetlands destroyed, dams built, crops = food provided, water sources polluted (nitrification, pathogens etc), poor biosecurity allowing comingling of domestic animals / birds and wildlife.

HUMAN POPULATION EXPANSION AND MINING:

Uncontrolled settlements and urban development, roads etc causes habitat destruction and pollution, increased interaction between wildlife, domestic animals and man, dams and water reticulation schemes provide new, and alter existing aquatic habitats, pollution of rivers and wetlands.

The concept of “ecohealth” highlights the interplay between agriculture, animal (domestic and wildlife) health, human health, ecosystem health and socio-cultural factors.

MODELLING HIGH RISK GEOGRAPHICAL AREAS IN SOUTH AFRICA IN TERMS OF MIGRATORY WILD BIRDS AND PANDEMIC HPAI.

In a landmark study conducted in 2007 by scientists from the Percy FitzPatrick Institute of African Ornithology, University of Cape Town, they present a simple, spatially explicit risk analysis for avian influenza transmission by wild ducks in southern Africa.(Cumming et al 2008).A risk value for each of 16 southern African anatid species was developed and summed risk estimates at a quarter-degree cell resolution for the entire subregion using data from the Southern African Bird Atlas. The risk variables considered include: Range, Abundance, Mobility, Mixed Flocks, Foraging and Anthropogenic association; calculated to a single "risk score" expressed as a %, per species. Not surprisingly Egyptian Geese and Spurwing Geese as well as the most common duck species; Red-Billed Teal, Yellow-Billed Duck and White Faced Whistling Ducks scored the highest ie they represent the highest risk in terms of AI potential spread. They then quantified environmental risks for South Africa at the same resolution. The risk variables for the environmental evaluation process included: Density of Chickens (based on data on commercial poultry production from 2006), Population Density (2001), Mean Monthly Temperature and Rainfall (1995), Coefficient of variation in mean monthly rainfall (1995), Total area as well as total perimeter of wetlands (2006) and Grains = area classified as irrigated crops or grasslands (2001).Here risks were expressed on a scale of 1=low to 5=high. Combining these two risk estimations produced a simple risk map for avian influenza in South Africa, based on the best currently available data. The areas with the highest risk values were those near the two largest cities, Johannesburg and Cape Town, although parts of Kwazulu-Natal and the Eastern Cape also had high-risk scores. Their approach has the virtue that it could be readily applied in other relatively low-data areas in which similar assessments are needed; and it provides a first quantitative assessment for decision makers in the subregion, as well as a platform to design further, focused surveillance efforts.

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AVIAN INFLUENZA IN COMMERCIAL POULTRY AND OSTRICH PRODUCTION SYSTEMS: EPIDEMIOLOGY AND SURVEILLANCE

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SUMMARY

From a global perspective, poultry farming encompasses not only the production of chickens and table eggs, but also turkeys, waterfowl and other avian species. Commercial poultry farming is a substantial vertically-integrated agribusiness in which local producers are dependant on a small number of international suppliers for genetic stock. There is significant cost-driven commodity-based international trade in poultry products from localities with low production costs to markets where demand exceeds local output. Global production of poultry products is expected to more than double over the next 30 years, primarily through the expansion of production capacity by multi-national corporations in developing countries with cheap access to the required inputs - specifically water for the production of crops, as drinking water for the birds and for use in the processing of product. The South African industry is largely focussed on chickens and eggs for regional consumption, with a smaller export-orientated ostrich industry.

Effective management of disease through biosecurity, vaccination and (where appropriate) medication is of utmost importance in order to maintain production efficacy and remain competitive. From a disease perspective Avian Influenza (AI) is one of the most significant threats, requiring active management on an ongoing basis. Whilst all birds could be considered to be vulnerable to AI, they vary considerably in their susceptibility to the virus. Wild waterfowl are regarded as the natural, usually asymptomatic, host within which any AI subtype could possibly circulate, potentially resulting in dissemination along the migratory flyways which these birds frequent on a seasonal basis. Gallinaceous birds, especially turkeys and chickens, are particularly sensitive to AI. In the classical epidemiological cycle, circulating AI viruses pass from wild waterfowl to farmed waterfowl. Subsequent exposure of highly susceptible farmed gallinaceous birds precipitates the emergence of either low pathogenic avian influenza (LPAI) involving any of the AI virus subtypes or alternatively, in the case of certain H5 and H7 subtypes, highly pathogenic avian influenza (HPAI). Whilst LPAI often compromises production efficacy in affected flocks, HPAI is a devastating disease resulting in extremely high mortalities in susceptible birds. Where LPAI involving H5 and H7 subtypes becomes problematical (especially in gallinaceous birds), mutation to HPAI can occur. Following primary exposure, rapid secondary dissemination often results in dramatic HPAI outbreaks with significant production losses requiring costly eradication programmes. During these outbreaks and control efforts human exposure to AI is a significant risk. Once controlled in farmed birds, HPAI classically disappears from exposed wild birds. Deviations from this classical epidemiological cycle, such as the evolution of H5N1 in Asia since 1996, need to be followed closely to understand the risk to humans, poultry and other animal species.

AI has been used as a model by the OIE, WHO and FAO to demonstrate the advantages of the One World One Health initiative. From the poultry perspective, the OIE has been instrumental in developing credible guidelines with regard to AI surveillance and management in order to create a risk-based framework within which the international trade in poultry commodities can continue.

INTRODUCTION

As the growth and urbanization of the human population continues, the need for the commercialisation and expansion of production capacity of basic foodstuffs grows proportionately. As a generalization, this urbanization is associated with increasing human social development and wealth. A noted consequence of increasing wealth is the trend to consume a higher proportion of protein of animal origin. As poultry is regarded as one of the cheapest freely available forms of animal protein, the international consumption is expected to double over the next thirty years.

Commercial poultry farming forms part of a global agribusiness incorporating the large-scale farming of chickens, turkeys, ducks and geese, as well as more exotic species such as ostriches. The typical commercial poultry farming structure consists of a small number of highly competitive large enterprises with significant vertical integration. Many of these enterprises operate multi-nationally, but all are dependant on a handful of international suppliers of genetic stock. From a trade perspective, there is a growing international flow of poultry-based commodities from regions where production costs are lower to regions where demand for these commodities outstrips the local ability to supply – either because of relatively high local production costs, or because of the scarcity key resources such as water and land. Most of the future expansion in capacity in the poultry industry is thus expected

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to occur in developing countries. Poultry farming enterprises focussed on local consumption typically having their production facilities concentrated around big cities where the demand for their products is greatest.

CHICKEN FARMING

The selected lines of chickens used for commercial broiler and layer farming differ to such an extent, that they should be viewed as separate categories of poultry. In both cases the production integration consists of three sub-sectors – the supply of genetic stock (pure lines), the multiplication phase (grandparent lines and parent lines) and the commercial stock.

In the table egg industry, there are about 6 billion hens in production in the commercial sector globally, with as many as 8 billion birds on the ground at any one time if the entire production integration is considered. The comparative figures for South Africa are 22 million commercial hens, with up to 30 million birds in the entire integration.

In the broiler industry, some 50 billion broilers are produced annually with 6 billion birds being on the floor at any one time in the complete production integration. South Africa's broiler sector produces some 900 million broilers per annum, with 100 million birds on the floor at any one time in the entire integration.

OTHER POULTRY FARMING

Whilst global duck farming is estimated to be about 3 billion birds per annum, South African production is negligibly small. Similarly, we no longer have a turkey industry of any significance, with global output being over 650 million birds per annum.

South Africa is however blessed with an ostrich industry, most of which is located in the Karoo. More recently, there has been expansion into the Eastern Cape and some other areas. In contrast to the chicken industry where most of the product is for local consumption, the ostrich industry is export orientated. Production systems as less well defined, but can loosely be divided into the breeding phase, the chick-rearing phase, the growing-out phase and the pre-slaughter quarantine phase. There are about 30 thousand breeding birds used to produce hatching eggs. Due to disease risks and the labour intensive nature of the chick-rearing phase, chicks tend to be distributed to smaller farmers to be cared for to 3-4 months. At this stage they are returned to bigger farmers who grow them out to about 12 months of age, either in feedlots or more extensively on open lands. It is estimated that some 250 thousand ostriches are slaughtered annually. Because the industry is export-orientated, the birds are quarantined for a period in preparation for slaughter in order to manage the risk of transmission of certain diseases of importance to international trade.

AVIAN INFLUENZA EPIDEMIOLOGY

Unfortunately, from the perspective of Avian Influenza (AI), the most widely farmed categories of poultry (chickens, turkeys and waterfowl) each have a special role to play in the epidemiology of AI. Both chickens and turkeys happen to be particularly susceptible to AI, whilst waterfowl are regarded as its natural asymptomatic host in the classical AI epidemiological cycle.

In the classical AI epidemiological cycle, AI viruses of diverse subtypes circulate in wild waterfowl. Seasonally, migratory waterfowl frequent well-defined global flyways along which these AI viruses co-mingle, with the more susceptible juvenile birds playing an important role in viral shedding and persistence. Incidental exposure of farmed waterfowl to these AI viruses results in circulation within farmed waterfowl with eventual extension to other farmed birds.

Whilst birds vary significantly in susceptibility, in the more susceptible gallinaceous birds such as chickens and turkeys, high levels of viral shedding can occur with an increased possibility for viral mutation. The consequence could either be a mild disease syndrome caused by any AI subtype termed low pathogenicity avian influenza (LPAI) or in the case of certain specific strains of the H5 and H7 subtypes a devastating syndrome termed highly pathogenic avian influenza (HPAI). Because of the regional concentration of poultry farms and the high shedding rates, secondary dissemination to other poultry is very common in outbreaks of AI. A host of potential routes of transmission have been implicated including, but not limited to:

1. Wild birds
2. Contaminated water
3. Contaminated feed
4. Other stock: poultry, pigs etc.
5. Humans
6. Fomites: on equipment, vehicles, bedding material etc.

AI outbreaks typically require huge efforts to bring under control, historically in the case of HPAI including costly eradication programmes, particularly for countries involved in the international trade in poultry commodities.

Deviations from this classical epidemiological cycle have been recorded. In 1961, on the South African coast, there was an unexplained outbreak of H5N1 HPAI in terns characterised by significant mortalities without involvement of farmed birds (Swayne and Halvorson (2003)). More recently, since 1996, the Asian H5N1 crisis has evolved into an intercontinental epornic involving not only farmed poultry, but also clinical disease with HPAI virus persistence in certain wild waterfowl species and probable HPAI dissemination by wild waterfowl (Alexander and Brown (2009)). In some parts of the world persistent LPAI circulation in farmed gallinaceous birds is emerging as a worrying concern, most notably of the H9 subtype in the Middle East and of the H6 subtype in a number of areas including Southern Africa.

The epidemiology of AI in ostriches is not well understood, but AI viruses of a variety of subtypes are known to circulate in ostrich populations as well as in the associated wild bird populations.

SURVEILLANCE

With the potential impact that the presence of HPAI in farmed poultry could have on the ability of a country to trade internationally, the OIE has been instrumental in setting an acceptable standard for HPAI surveillance.

In South Africa, due to the focus on production for local consumption with regard to the chicken industry, surveillance is largely focussed on confirming the continued absence of HPAI in commercial chicken farms. As a consequence, all commercial farms are required to submit 30 blood samples bi-annually for serological monitoring. Limited serological surveillance is also conducted within informal poultry. The testing regime employed involves the use of a group-specific AI ELISA for screening, followed by a haemagglutanin-specific HI test again H5 and H7 antigens for positive samples.

For chicken enterprises involved in the export of commodities of chicken origin, a system of compartmentalisation with more stringent testing (depending on the requirements of the importing countries, but typically involving monthly testing) was established, but has now largely fallen into disuse.

As opposed to chickens, with the focus on exports of ostrich commodities, surveillance in the ostrich population is far more intensive. Besides the bi-annual HI testing of 30 samples per epidemiological unit for the H5 and H7 subtypes, a system of serological monitoring prior to intended movement has also been established for juvenile birds. Prior to slaughter for export ostriches are quarantined and individually tagged, with 30 samples per flock being tested serologically for H5 and H7 AI subtypes during this period.

DISCUSSION

With the emphasis in poultry on the H5 and H7 subtypes of AI virus and the fact that these may not necessarily be the most significant from the perspective of human health, it is vitally important that initiatives such as One World One Health are persisted with and indeed expanded to include other diseases. Human exposure to AI viruses has in a number of cases resulted in infection (Peiris (2009)). It must be noted that if in the case of chickens one assumes the involvement of at least one human for every 40 thousand birds, with 8 billion layer-type birds and 6 billion broiler-type birds on the ground at any one time there are more than 350 thousand people in intimate daily contact with poultry globally. The fact that LPAI AI viruses circulate regionally in many parts of the world reflects on the inability of the poultry industry to manage the exposure of flocks to AI viruses in general. From a human health perspective, some of these LPAI viruses, more specifically of the H9 subtype, may be of more importance to human health than the HPAI viruses.

With regard to improving our understanding of the ongoing HPAI problems in Asia (Sims, L. D., Domenech, J., Benigno, C., Kahn, S., Kamaya, A., Lubroth, J., Martin, V. and Shortridge, K. F. (2003)), it is important to note that the bulk of the global commercial farming of waterfowl in fact takes place in this region – this may be one of the pivotal differences in the region making HPAI control so difficult and inconsistent with the classical epidemiological cycle.

Although the epidemiology of AI in ostriches has been investigated to some extent (Thompson, P. N., Sinclair, M. and Gaanzevoort, B. (2008)), from a South African perspective it deserves more attention. The HPAI outbreaks in ostriches in South Africa are notable from the perspective that differing HPAI viruses have emerged in these cases without the apparent involvement of other poultry. In the South African cases it would appear that affected ostriches do not seem to be notable shedders or multipliers of AI virus and could possibly be reduced to being “innocent bystanders”.

HPAI CONTROL

Key elements to any attempt to control an HPAI outbreak include:

1. Thorough preparedness for any outbreaks
2. Prompt recognition of any outbreaks
3. Rapid response to any outbreaks
4. Protecting food security following resolution of any outbreaks

Appropriate biosecurity, including measures pertaining to both bio-exclusion (keeping pathogens out of unaffected flocks) as well as bio-containment (preventing pathogens from spreading from affected flocks) needs to be in kept in place for the national flock. In order to ensure that adequate resources can be allocated timeously to a potential HPAI outbreak, private-public partnerships have to be established beforehand including experts from appropriate fields. It is vitally important that national and regional contingency plans are not only in place, but have also been tested through "dry runs" which have been independently audited to ensure that they are robust. Any HPAI outbreak would put enormous pressure on available recourses – only becoming aware of weaknesses in contingency plans in the midst of a real crisis would be potentially catastrophic and could result in the inability to bring such an outbreak under control.

Effective surveillance and diagnostic systems using standardised testing regimes such as those established by the OIE in approved laboratories are essential to making a prompt diagnosis. Farmers and veterinarians need to be comfortable to make use of such facilities should HPAI potentially be suspected. A key element in achieving this pertains to a very clear compensation policy agreed to by farmers well before it could be needed (Anon. (2006)). Delays in reporting suspected cases due to uncertainty as to the consequent outcome for the affected farmers have repeatedly been seen to be important in determining the success of eradication efforts.

Once an outbreak has been confirmed, a response team of appropriately qualified experts with the ability to take the firm and often difficult necessary decisions timeously in order to allow control efforts to succeed needs to be in place. Unnecessary procrastination could allow initial small primary cases to explode into potentially uncontrollable secondary outbreaks well before the necessary controls are agreed to.

Due to the dramatic impact on mortalities in susceptible flocks as well as the need to carry out eradication in affected zones HPAI poses a potential risk to food security not only on a national basis, but regionally as well. Ensuring effective decontamination prior to restocking is vitally important to ensuring a rapid recovery in production to limit this risk to food security. The way in which the global poultry industry has operated historically, with each breeding company concentrating its pure lines at a single facility at a "safe" location has been questioned following the dissemination of the Asian H5N1 HPAI across Europe in recent years. Breeding companies have now established alternative centres from which genetic stock can be sourced on more than one continent.

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EVIDENCE OF INFLUENZA A VIRUS INFECTION IN CAPTIVE BIRDS AT NATIONAL ZOOLOGICAL GARDENS OF SOUTH AFRICA, PRETORIA

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SUMMARY

Influenza A viruses are widespread in wild birds, particularly waterfowl, and do not usually cause disease in these natural hosts. However, when the viruses are transmitted to susceptible terrestrial hosts, particularly poultry, they may cause avian influenza, an OIE-listed disease. The highly pathogenic forms of the disease, caused by the H5 and H7 subtypes, have zoonotic potential. Surveillance of avian populations therefore forms an important early warning system for this potentially devastating disease. Zoos, wildlife sanctuaries, rehabilitation centres and similar institutions where different bird species occur in the same place and are at the same time exposed to free-ranging wild birds, form ideal sentinel populations to monitor and perform AI surveillance. During 2008-2009, free-ranging, captive endemic and exotic birds (233 birds from 58 different species) from the National Zoological Gardens of South Africa (NZG) in Pretoria were swabbed (cloacal and tracheal) and tested for AIV, using real-time reverse transcription polymerase chain reaction (rRT-PCR). Additionally, pooled samples from 70 and 142 Rosy flamingos (*Phoenicopterus ruber*) were collected during 2008 and 2009 respectively and tested for Avian Influenza Virus (AIV) and Newcastle Disease Virus (NDV) using real-time RT-PCR. Out of 455 swab samples tested, 52 (11.5%) tested positive for AIV, mostly from exotic birds. Seven pooled Rosy flamingo samples collected during 2009 tested positive for exposure to AIV, and three of these pools were confirmed positive for NDV using a hemi-nested conventional RT-PCR assay.

INTRODUCTION

Avian influenza (AI) is caused by Influenza A virus, a member of the *Orthomyxovirus* family (single stranded, negative-sense, segmented RNA genome viruses). AI is a contagious disease of domestic fowl, migratory and resident water birds, pigs and occasionally humans, making this notifiable zoonotic infection one of the most important animal diseases (Capua and Alexander (2006)). Avian influenza virus (AIV) infection can have serious medical, economical and epidemiological implications including siege on international trade, hesitance in consumer confidence, and the cost associated with eradication (Munch *et al*. (2001); Hlinak *et al*. (2006)) Pedersen *et al*. (2010)). Infections in birds have been associated with a variety of disease syndromes ranging from sub-clinical to mild upper respiratory disease, loss of egg production, and acute generalized fatal disease (Xie *et al*. (2006)). Waterfowl and shorebirds, however, are believed to be natural reservoirs and asymptomatic carriers of essentially all sixteen hemagglutinin and nine neuraminidase combinations of influenza A virus (Reed *et al*. (2002); Lupiani & Reddy (2005); Stürm-Ramirez *et al*. (2004); Chen *et al.* (2006); Hlinak *et al*. (2006)). The H5 and H7 serotypes are prone to mutation in terrestrial poultry to the pathogenic notifiable forms of the disease, and in recent years a notifiable (HPNAI) H5N1 strain has arisen that is capable of causing morbidity and mortality in numerous wildlife species (Reed *et al*. (2002); Alexander, (2008)).

Newcastle disease (NCD) causes similar symptoms to AI and the virulent forms are also notifiable to the World Animal Health Organisation (OIE). The aetiological agent is a member of the *Paramyxoviridae* family (single-stranded, negative sense, non-segmented RNA genomes) commonly known as Avian Paramyxovirus Type I or Newcastle disease virus (NDV). Unlike AIV, only a single serotype of NDV is known (Mayo, (2002)) but several lineages are described (1 to 6), of which lineage 6 (also known as class I) infect wild birds asymptomatically (Czegledi *et al*., 2006).

The National Zoological Gardens of South Africa (NZG) houses an important sentinel avian population. It is envisaged that viruses are transmitted through the interaction of a variety of free-ranging birds and captive endemic and exotic birds. The primary aim of this study was to determine the prevalence of AIV infections in NZG birds in the late summer through spring months (April/ May to October) in both 2008 and 2009.

MATERIALS AND METHODS

SAMPLES

Tracheal and cloacal swabs were taken from birds submitted for clinical or necropsy examination at the NZG, between the period May to August of 2008 and April to October 2009 (n=445) (Table 1). Birds submitted for necropsy examination from outside the Zoo were also tested. A list of species tested follows below. Swabs were stored in viral transport medium (50% glycerol: 50% phosphate-buffered saline, pH 7.2., containing penicillin, streptomycin and amphotericin B (Lonza, BioWhittaker®)) at -20°C

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prior to RNA extraction. Blood samples were collected from four Rosy flamingos (*Phoenicopterus ruber*) for serological detection of AIV exposure.

Endemic pscittacines (n=2)
Brown Headed Parrot (*Poicephalus paradiseus* n=2)

Exotic pscittacines (n=69)
African Gray (*Psittacus erithacus* n=5); Amazon (*Amazona amazonica* n=2); Blue and Yellow Macaw (*Ara ararauna* n=2); Cacatua Leadbeater (*Lophochroa leadbeateri* n=2); Greater Jardine (*Poicephalus guliemi massaicus* n=2); Kea (*Nestor notabilis* n=2); Little Carella (*Cacatua sanguinea* n=2); Nandaya Conure (*Nandayus nenday* n=10); Rainbow Lorikeet (*Tricholossus haematodus* n=24); Red Fronted Macaw (*Ara rubrogenys* n=2); Red-sided Eclectus (*Ara rubrogenys* n=2*)* Scarlet Macaw (*Aramacao* n=2); Solomon Eclectus (Eclectus roratus n=2); Sun Conure (*Aratinga solstitialis* n=6); Yellow Streaked Lorikeet (*Chalcopsitta cardinalis* n=4).

Endemic raptors (n=27)
Barn Owl (*Tyto alba* n=6); Black Shouldered Kite (*Elanus axillaris* n=2); Brown Eagle (*Circaetus cineareus* n=2); Cape Vulture (*Gyps coprotheres* n=3); Giant Eagle Owl (*Bubo lacteus* n=4); Scops Owl (*Otus scops* n=4); Spotted Eagle Owl (*Bulbo africanus* n=2); White Faced Owl (*Ptilopsis granti* n=2); White Backed Vulture (*Gyps africanus* n=2);

Exotic raptors (n=10)
Black Spotted Owl (*Strix occidentalis caurina* n=2); King Vulture (*Sarcoramphus papa* n=8);

Endemic waterfowl (n =11)
Egyptian Geese (*Alopochen aegyptiacus* n=7); Red Knobbed Coot (Fulica cristata n=4);

Exotic waterfowl (n=224)
Black Swan (*Cygnus atratus* n=4); Knob-Billed Duck (*Sarkidiornis melanotos* n=2); Mute Swan (*Cygnus olor* n=2); Rosy Flamingo (*Phoenicopterus ruber* n=212); Spoonbill (*Platalea alba* n=4).

Other endemic species (n= 84)
Blue Crane (*Anthropoides paradiseus* n=2); Cattle egret (*Bubulcus ibis* n=2); Domestic Chicken (*Gallus gallus domesticus* n=2); Glossy Starling (*Lamprotornis purpureus* n=2); Crowned Plover (*Vanellus coronatus* n=2); European Roller (Coracias garrulus n=2); Feral Pigeon (Columbiae livia domestica n=12); Goliath Heron (*Araea galiath* n=2); Green Pigeon (*Treron phoenicoptera* n=4); Grey Lourie (*Coryathai sdoides concolor* n=4); Hadeda Ibis (*Bostrychia hagedash* n=8); Laughing Dove (*Streptopelia senegalensis* n=4); Pied Crow (*Corvus albus* n=2); Red Billed Oxpecker (*Buphagus erythrorhynchus* n=4); Purple Crested Touraco (*Tauraco porphyreolophus* n=8); Red Eye Dove (*Streptopelia semitorquata* n=12); Scarlet Ibis (*Eudocimus ruber* n=4); Speckled Mousebird (*Colius striatus* n=2); Spotted Dikkop (*Burhinus capensis* n=2); Star Finch (*Neochmia ruficauda* n=2); Swainson's Francolin (*Francolinus swainsonii* n=2)

Other exotic species (n=17)
Bald Ibis (*Geronticus calvus* n=5); Helmeted Guinea Fowl (*Numida meleagris* n=4); Keel Billed Toucan (*Ramphastos sulfuratus* n=2); Toco Toucan (*Ramphastos toco* n=2); Turkey (*Meleagris ocellata* n=2); Wattled Crane (*Bugeranus carunculatus* n=2).

RNA EXTRACTION AND AIV REAL-TIME REVERSE TRANSCRIPTION- POLYMERASE CHAIN REACTION (RRT-PCR)

RNA extraction was performed using TRIzol® LS Reagent (Invitrogen) according to the recommended procedure. 2ul of RNA was tested for the presence the AIV group (matrix protein gene target) with a TaqMan® Influenza A Detection 2.0 kit (Applied Biosystems) on a Step One Plus thermal cycler (Applied Biosytems) according to recommended protocol. Influenza A positive cases were further tested for the presence the H5 and H7 subtypes using the respective TaqMan® Influenza H5/H7/N1 Detection 2.0 kits (Applied Biosystems). In interpretation of the results, both the Ct value (cycle threshold) which is defined as the number of cycles required for the fluorescent signal to cross the threshold as well as the relative position of the test sigmoidal fluorescent curve in relation to those of the positive and negative controls were considered. Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

NDV REAL-TIME REVERSE TRANSCRIPTION- POLYMERASE CHAIN REACTION (RRT-PCR) AND NESTED CONVENTIONAL RT-PCR

RNA was tested for the presence of NDV nucleic acid (group-specific L gene target) using the method described by Fuller *et al.* (2010) with a QuantiFast™ Probe RT-PCR kit (Qiagen) on a Light Cycler 480 thermal cycler (Roche). Positive reactions were

confirmed using a conventional hemi-nested RT-PCR assay that additionally distinguishes avirulent from virulent NDV subtypes (Wang *et al*., 2001).

VIRUS ISOLATION

Positive material were submitted to ARC-OVI's Virology Section for inoculation into embryonated fowl's eggs according to standard OIE-recommended procedure (2009), however no viruses were isolated.

ENZYME-LINKED IMMUNO-SORBENT ASSAY (ELISA) FOR THE DETECTION OF ANTI-INFLUENZA ANTIBODIES

A limited number of flamingo serum samples (n=4) were tested for the presence of anti-AIV group antibodies using a competitive assay, viz. the AI MultiSScreen ELISA kit (IDEXX), according to the recommended procedure. Sample to Negative (S/N) ratios were calculated as recommended in the protocol.

RESULTS

AIV PREVALENCE

52 samples out of 455 (11.7%) tested positive for AIV, although none of these were positive for the H5 or H7 subtypes (Table 1). The flamingos represented the highest prevalence of the positive cases by far (35/52, or 67.3%), followed by the raptors (6/52, 11.5%) and the pscittacines (4/52, 7.7%) with the other species making up the remaining 11.5%. Tracheal swab positives were more frequently detected than cloacal swab positives, whereas this was most notable for the flamingo group where only the tracheal swabs tested positive. As indicated in Table 1, not all the positive cases were resident or trapped at NZG in Pretoria. Year by year, 6/30 (20%) were positive in 2008, whereas 45/455 (10.1%) tested positive for AIV in 2009 (Figure 1). In 2008 positives were only detected in May, whereas the positive cases were more evenly distributed during the 2009 sampling period.

NDV PREVALENCE IN ROSY FLAMINGOS

Flamingo samples collected in 2009 in 28 pools were additionally tested for the presence of NDV, initial screening was done with rRT-PCR and 7/28 (25 %) of these pools tested positive (Table 2). Confirmatory testing and pathotyping using a conventional hemi-nested RT-PCR identified three of the pools (7, 8, 27) as NDV positive. Results are indicated in Fig. 3. All three positive pools were positive for avirulent NDV, but pools 7 and 27 were also positive for virulent NDV.

AI ELISA RESULTS FOR ROSY FLAMINGOS

Four random blood samples collected from Rosy flamingos were tested by a non-species specific AI ELISA, results are indicated in Table 3. Samples with an S/N ration ≥ 0.50 are considered negative for the presence of AI antibodies. Samples with S/N value < 0.50 should be considered AI antibody positive. No anti-AI antibodies were detected.

DISCUSSION

455 samples representing 58 species that were resident, wild or submitted dead to the NZG in Pretoria were tested for AIV between April and August in 2008 and 2009. An overall AIV incidence of 11.7 % was observed, which is considerably higher than the observed incidence of <1% detected in free-living wild birds in South Africa surveyed over the same period (data to be published elsewhere). The Rosy flamingo population in particular had a high incidence of AIV (67.3%), although four blood samples tested negative for exposure. This could be explained by a recent exposure to the virus as indicated by the presence of viral nucleic acid, whereas the antibody response typically takes longer to develop. Since the blood sample represented a very small proportion of the population, it is not possible to draw a meaningful conclusion from the serological results. The flamingos were also tested for NDV, and 25% of the pools tested positive. Interestingly, a feral pigeon captured in the flamingo enclosure also tested positive for AIV and NDV. Columbiformes gather in large numbers at Flamingo feeding sites. The flamingo enclosure with its open water attracts many wild waterfowl as well as passerine species, and may well represent the ideal sentinel population within the NZG. Since flamingos are filter feeders, their higher viral exposure could be linked to their feeding behaviour, although this is speculative.

Conventional hemi-nested RT-PCR results were ambiguous (avirulent bands were observed for all three positive cases but virulent bands were also present in two of these). These three positive samples were further tested for pigeon paramyxovirus (NDV lineage 4; Abolnik *et al*. (2008)) using a specific primer in the hemi-nested RT-PCR, but the results were negative (data not shown). Attempts at characterising the positive samples through DNA sequencing are ongoing.

AIV and NDV are spread via the fecal-oral route, thus as wild birds perch on top of enclosures, droppings can contaminate feed and water, and birds attracted into open enclosures can transmit viruses in the same manner. Mechanical transmission (personnel and equipment) moving between infected enclosures and cages would also be a risk factor once infection is established. The presence of virus in mainly captive Psittacines and captive and free-ranging raptors rather than water birds is of considerable interest; as is the finding of positive free-ranging Red billed Oxpeckers and a Laughing Dove. This warrants further research; continuing annual surveillance should also provide additional valuable information

CONCLUSION

The captive birds of zoological gardens are ideal sentinel populations for viruses such as AIV and NDV and we therefore monitor the NZG population as part of the national surveillance programme. The programme is intended to act as an early warning system for the incursion of pathogens that threaten the poultry and human populations, such as HPAI H5N1. NZG bird and animal collections are however valuable in their own right for conservation purposes, and must be protected from incursion of potentially devastating diseases as far as possible. The deaths of zoo tigers fed infected chicken carcasses in Thailand during the HPAI H5N1 epidemic (Amonsin *et al*. (2006)) illustrates this point. Biosecurity awareness is important: open feed and water must be protected from wild birds where possible, regular disinfection of feed and water troughs must be done and care should be taken to avoid mechanical transmission of pathogens between enclosures (for example, the disinfection of implements and boots). Efforts at detecting and characterising both AI and ND viruses in NZG collection will continue, as we refine our surveillance strategies.

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Table 1: AIV Real-Time RT-PCR Positive cases for the year 2008-2009

Collection Date	Species	# Positives/ total	Swab sample	Origin	AIV M gene rRT-PCR Ct Value	AIV H5 / H7 rRT-PCR results	Remark
05/2008	Cape Vulture (*Gyps coprotheres)*	1/3	Tracheal	NZG, Encl. 46	33.94	Negative	Necropsy
05/2008	King Vulture (*Sarcoramphus papa)*	1/8	Tracheal	NZG, backyard	33.5	Negative	Alive Donated: Mokopane
05/2008	King Vulture (*Sarcoramphus papa)*	1/8	Cloacal	NZG, backyard	33.43	Negative	Alive Donated: Mokopane
05/2008	Bald Ibis (*Geronticus calvus)*	1/5	Tracheal	NZG, Encl. 217	32.63	Negative	Necropsy
07/2008	Laughing dove *(Streptopelia senegalensis)*	1/4	Tracheal	NZG, Free-ranging	31.85	Negative	Alive
07/2008	Red-sided eclectus (*Ara rubrogenys)*	1/2	Cloacal	NZG, Encl. 303	30.70	Negative	Necropsy
05/2009	Jendaya Conure (*Nandayus nenday)*	1/10	Tracheal	NZG, Encl. 24	31.42	Negative	Necropsy
05/2009	Red-fronted macaw (*Ara rubrogenys)*	1/2	Cloacal	Found dead	28.7	Negative	Natal
05/2009	Spotted Eagle owl (*Bulbo africanus)*	1/2	Tracheal	Found dead	33.02	Negative	Hit by car up on N14 Highway
05/2009	Spotted Eagle owl (*Bulbo africanus)*	1/2	Cloacal	Found dead	32.35	Negative	Hit by car up on N14 Highway
06/2009	Cape Vulture (*Gyps coprotheres)*	1/3	Tracheal	Found dead	22.24	Negative	Brits
07/2009	Rosy Flamingo (*Phoenicopterus rubber*)	35/212	Tracheal	NZG, Encl. 42		Negative	Alive
07/2009	Sun Conure (*Aratinga solstitialis)*	1/6	Tracheal	NZG, Encl. 24	31.00	Negative	Necropsy
07/2009	Feral Pigeon (*Columbidae livia domestica*)	1/12	Tracheal	NZG, Encl. 42	29.06	Negative	Trapped at Enclosure 42
08/2009	Red-billed oxpecker (*Buphagus erythrorhynchus)*	1/4	Cloacal	Found dead	16.25	Negative	Mokopane/caught 20/7 in KNP
08/2009	Purple-crested touraco (*Tauraco porphyreolophus)*	1/8	Cloacal	Found dead	24.78	Negative	Donation: unknown location
09/2009	Kea *(Nestor notabilis)*	1/2	Tracheal	Found dead	26.64	Negative	Donated: unknown location
09/2009	Egyptian Goose (*Alopochen aegyptiacus)*	1/7	Tracheal	Found dead	26.18	Negative	Found in Pretoria Central

Table 2: Rosy Flamingo (Phoenicopterus ruber) NDV rRT-PCR and hemi-nested RT-PCR positive results

Pool	Swab samples	PCR results		
		rRT-PCR	Conventional hemi-nested RT-PCR	
		Ct values (positive)	Avirulent	Virulent
7	Cloacal	33.18	Weak positive	Strong positive
8	Cloacal	34.43	Strong positive	Negative
17	Tracheal	34.8	Negative	Negative
19	Tracheal	33.98	Negative	Negative
22	Tracheal	34.26	Negative	Negative
26	Tracheal	34.16	Negative	Negative
27	Tracheal	32.76	Strong positive	Weak positive

Table 3: Flamingo serum AI ELISA results

	A(650) reading 1	A(650) reading 2	S/N ratio	Interpretation
Positive Control	0.398	0.468	0.32	Positive
Negative Control	1.283	1.416	1	Negative
Flamingo # 1	1.004	0.948	0.72	Negative
Flamingo # 2	1.091	1.045	0.79	Negative
Flamingo # 3	1.033	0.942	0.73	Negative
Flamingo # 4	0.989	1.072	0.76	Negative

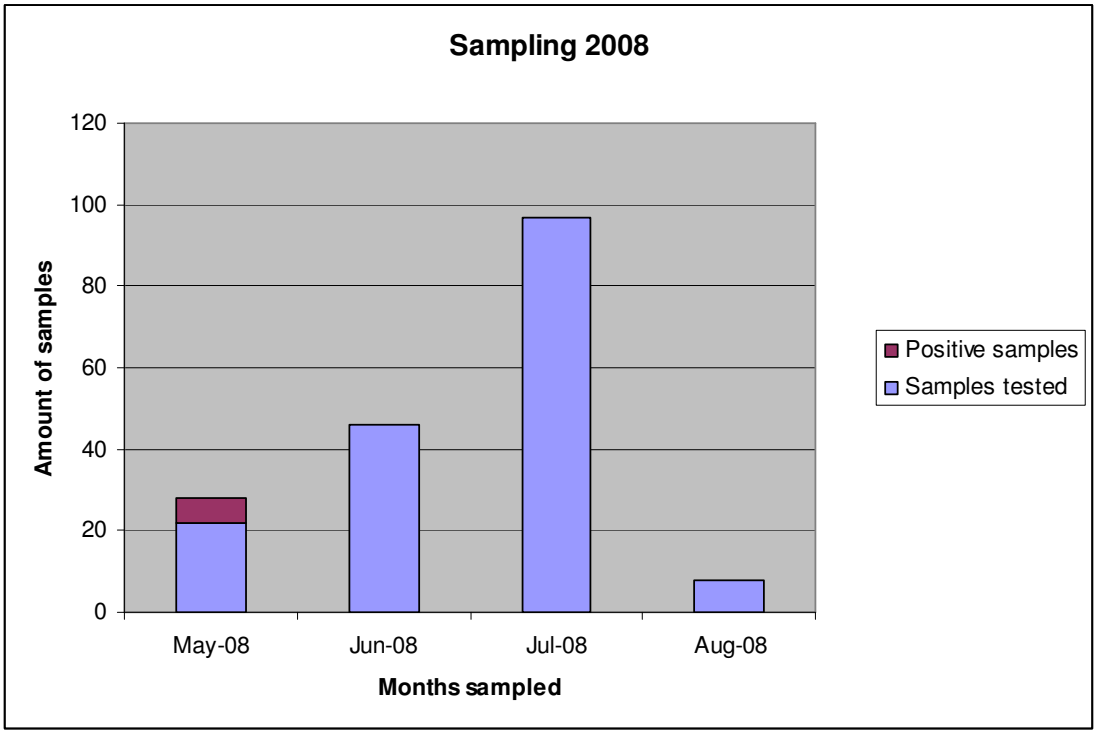

Figure 1: Frequency of positive AIV rRT-PCR detected per month in 2008

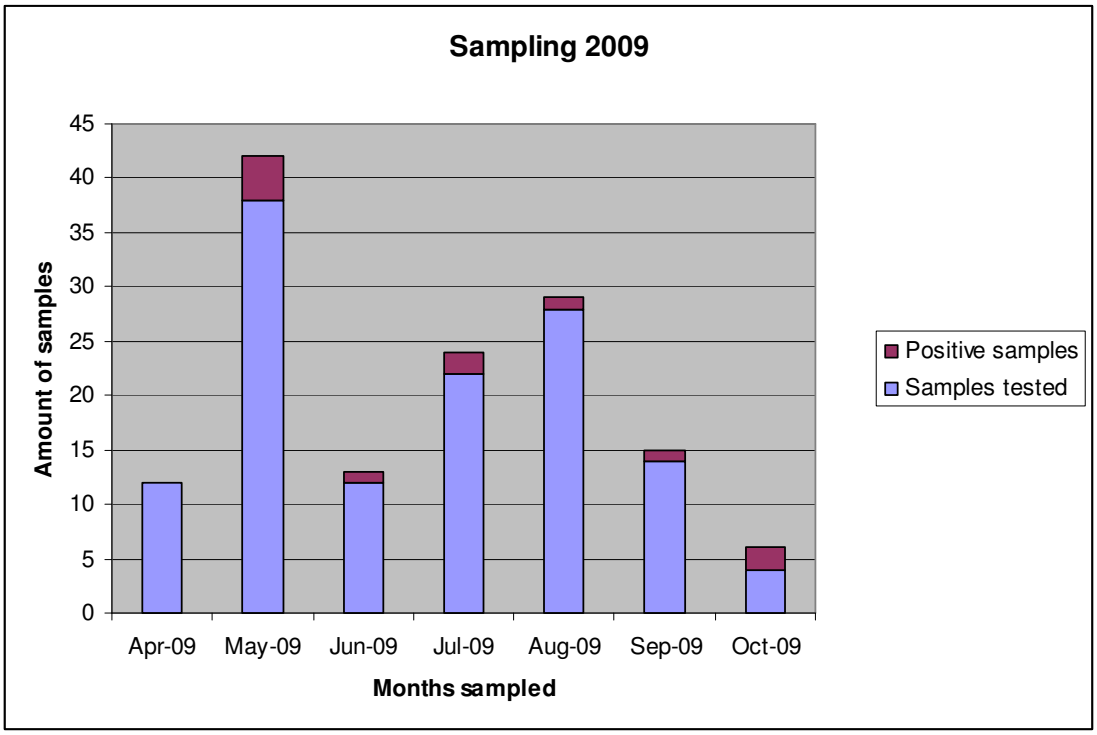

Figure 2: Frequency of positive AIV rRT-PCR detected per month in 2009

Figure 3: Hemi-nested RT-PCR results (1.5% agarose gel)
The position of the arrow indicates the expected amplicon size of 254bp.

Lanes 2-11: Nested Avirulent hemi-nested RT-PCR
Lane 1: 100 bp DNA marker
Lane 2: Pool 7
Lane 3: Pool 8
Lane 4: Pool 17
Lane 5: Pool 19
Lane 6: Pool 27
Lane 8: NDV Komarov strain (virulent) negative control*
Lane 9: NDV LaSota strain (avirulent) positive control
Lane 11: water negative control

Lanes 15-21: Nested Virulent hemi-nested RT-PCR results
Lane 14: 100bp DNA marker
Lane 15: Pool 7
Lane 16: Pool 8
Lane 17: Pool 17
Lane 18: Pool 19
Lane 19: Pool 27
Lane 21: NDV Komarov strain (virulent) positive control
Lane 22: NDV LaSota strain (avirulent) negative control
Lane 23: water negative control

*Primer design is not optimised for the Komarov strain, non-specific priming sometimes observed (unpublished laboratory data).

SWINE INFLUENZA IN COMMERCIAL PIG PRODUCTION SYSTEMS

T.A. Gous[1]

SUMMARY

Swine influenza is a significant cause of respiratory disease in pigs in North and South America, Europe and Asia. On the African continent, the disease has been reported in Kenya but no confirmed cases have been identified in pigs in southern Africa. Freedom from swine influenza (H1N1) in the national commercial South African pig herd was confirmed via serological surveys in 2000, 2004, 2007 and 2009. The disease is caused by Influenza A virus with H1N1 being the most common subtype. This subtype was first isolated in the USA in 1930. Numerous other subtypes have also been detected in pigs. Pigs are susceptible to infection with both human and avian influenza A viruses. This characteristic makes pigs ideal mixing vessels for the genetic reassortment of human/avian/swine influenza virus subtypes that can result in the emergence of novel human influenza viruses such as the 2009 pandemic H1N1 subtype. The clinical signs of influenza in pigs are remarkably similar to the symptoms in humans, and are typically characterised by an abrupt onset and short course of fever, anorexia, lethargy, coughing, dyspnoea, and nasal discharge. Morbidity tends to be high and mortality low (1-4%). Influenza viruses also act synergistically with other viral or bacterial pathogens to cause porcine respiratory disease complex. The course and severity of an influenza virus infection in a pig is influenced by management, intercurrent infections, age, immunity and virus subtype involved. The virus is spread among pigs by aerosols, direct and indirect contact and subclinically infected carrier pigs. Biosecurity and vaccination are the primary preventative measures.

INTRODUCTION

The isolation of a novel strain of swine-origin H1N1 influenza A virus in humans from Mexico and USA in April 2009, now known as pandemic (H1N1) 2009 virus (pH1N1)[7], and its rapid worldwide spread among humans, has recently put the spotlight on the global commercial pig industry.

Global meat consumption is increasing steadily. In 1983 the world consumed 152 million tons of meat per year. By 1997 consumption was up to 233 million tons and the FAO estimates that by 2020 world consumption could reach 386 million tons[9]. According to 2009 statistics of the United States Department of Agriculture, Foreign Agricultural Service, pork is the world's most widely eaten meat. Of all meat consumed on a global scale, 40% is pork, 29% chicken, 24% beef and 7% other[2].

There are in the region of 1 billion pigs in the world today compared to about 750 million 30 years ago. The top 10 pork-producing countries in the world are in decreasing order: China (49%), EU-27 (22%), USA (10%), Brazil, Russia, Vietnam, Canada, Japan, Philippines and Mexico. Approximate pig numbers for the top four countries are: China 500 million (50 million sows), EU-27 160 million, USA 60 million and Brazil 30 million. The average annual per capita pork consumption in the main pig producing countries is as follows: China 36.4 kg, EU-27 42.1 kg, USA 29.3 kg. The average South African consumes 3.7 kg of pork per annum (compared to 27.9 kg broiler meat)[2].

Over the last two decades there have been significant structural changes in the pig industry. Small mixed-production farming operations that produced several different crops and utilized pigs to consume by-products or excess grain have given way to large industrial farrow-to-finish units that are dedicated to pig production. For example, in 2001 in the USA about 70% of the total annual pork production was controlled by only 50 producers, of which 28% was controlled by the biggest 10 producers. The biggest US operation has over 710,000 sows. These industrial units are now expanding into Mexico, Brazil and eastern Europe where there are less stringent laws regarding environmental regulations, cheaper labour, fewer production constraints and therefore a decrease in cost of production[3,15].

The South African pig industry is minute compared to the global pig industry. There are 400 commercial pork producers in South Africa and they manage 103,000 sows that produce 157,000 kg of pork annually. Units vary from small farms with 50 sows to larger units with up to 7,000 sows. Approximately 250 units (all with more that 500 sows) produce 80% of the country's pork. Similar to the rest of the world, the trend in South Africa is for units to increase in size with smaller units being absorbed into the bigger ones. In total, 2.1 million pigs are slaughtered every year of which 45% is channelled to fresh meat markets and 55% to the processing sector. South Africa imports in the region of 23,000 kg pork per year. Almost 70% of these imports consist of ribs. Europe, Canada and Brazil are the main exporters to South Africa. Currently South Africa only exports a small quantity of pork to countries in Africa[24].

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HISTORICAL OVERVIEW

The most common subtypes of influenza A viruses in pigs are H1N1, H3N2 and H1N2. These subtypes are endemic in pig populations in North and South America, Europe and Asia, and are responsible for a highly contagious respiratory disease in pigs. Despite the same subtype classification, swine influenza viruses in Europe and the USA are genetically distinct[5].

Clinical disease consistent with influenza in pigs was first observed in 1918, coinciding with the "Spanish flu" pandemic in humans. The aetiological agent was influenza A virus of the H1N1 subtype, referred to as classical swine influenza virus, that was isolated in the USA in 1930[22]. Since then it was suspected that the virus could be transmitted from pigs to humans, which was confirmed in 1976 when virus was isolated from pigs and their caretaker on a Wisconsin farm in the USA[11]. Classical H1N1 viruses have also been isolated from pigs in South America, Europe and Asia[5]. The H1N1 subtypes in the USA remained antigenically and genetically highly conserved until the 1990's when antigenic and genetic variants started emerging[19].

There is only limited evidence for maintenance of human H1N1 influenza viruses after natural introduction into pig populations, but human H3N2 viruses have been recovered frequently from pigs in Asia and Europe[5]. The introduction of a human H3N2 subtype into the USA pig population during the 1990's was a critical factor in the emergence of the reassortant viruses that now dominate the pig population in that country[18].

There have been at least three independent introductions of distinct, wholly avian, H1N1 viruses to pigs[5]. In particular, an avian H1N1 virus introduced into pigs in Europe in the late 1970's spread throughout much of the continent and eventually became a dominant cause of swine influenza[6]. Serum antibodies against avian H4, H5 and H9 viruses have been detected in pigs in China; avian H1N1, H3N2 and H9N2 viruses have been isolated from Asian pigs; and avian H4N6, H3N3 and H1N1 viruses have been isolated pigs in Canada[18].

The segmented nature of the influenza genome allows two viruses that co-infect a single host to exchange RNA segments during viral replication. Pigs are susceptible to infection with both human and avian influenza A viruses because their respiratory epithelial cells express both the SA-α2,6-Gal receptors that are preferentially used by human influenza viruses and the SA-α2,3-Gal receptors preferred by avian influenza viruses[14]. This characteristic makes pigs ideal mixing vessels for the genetic reassortment of human/avian/swine influenza virus subtypes that can result in the emergence of novel influenza viruses[5,17]. Several of these reassortant viruses have been isolated from pigs since the late 1990's, including reassortant H3N2, rH1N2, rH1N1, rH1N7, rH3N1[18] and recently the 2009 pandemic H1N1 subtype[13].

In January 2010 a novel reassortant H1N1 subtype [A/swine/Hong Kong/201/2010 (H1N1)] was isolated from a pig at a Hong Kong abattoir during routine virological surveillance for influenza viruses. This novel virus is significant in that it contains a pH1N1-like NA gene, indicating a reassortant progeny of pH1N1 with swine viruses. This finding emphasises the importance of ongoing monitoring of pig populations for the emergence of novel reassortant and possible pandemic swine influenza viruses that could be transmitted to humans[25].

THE 2009 H1N1 PANDEMIC

The pH1N1 was soon after its first isolation in April 2009 designated as "swine flu" virus although it was never detected in pigs until the occurrence in a pig herd in Alberta, Canada in May 2009[13]. Some of the other countries with confirmed cases of pH1N1 in pigs include China[25], Argentina[20], Norway[12], Australia[1], Mexico, USA, Ireland, United Kingdom, Indonesia and Japan[16]. Swine influenza is not currently an OIE listed disease so pH1N1 may be more widespread than reports indicate.

The pH1N1 is a reassortant virus with at least three origins. Six of the genes are closest in sequence to those of H1N2 "triple-reassortant" influenza viruses isolated from pigs in North America around 1999-2000. The other two genes are from different Eurasian "avian-like" viruses of pigs - the NA gene is closest to H1N1 viruses isolated in Europe in 1991-1993, and the MP gene is closest to H3N2 viruses isolated in Asia in 1999-2000. The phylogenetic information presently available does not identify the source of pH1N1 but the possibilities mooted include unsampled pig herds, the intercontinental live pig trade, porous quarantine barriers, virology laboratories and vaccine production[10].

Recent studies have shown that pigs can be readily infected with pH1N1 resulting in viral transmissibility, pathogenesis, pathology and clinical signs that are similar to typical swine influenza viruses. These studies have also shown that the virus is fully capable of becoming established in global pig populations[4].

SWINE INFLUENZA THE DISEASE

Transmission of the virus is generally by direct contact between infected or subclinical carriers and susceptible individuals, although infected fomites may also be a source of infection. Humans and birds may also act as a source of infection. Virus spreads from pig to pig by snout to snout contact, by aerosol or droplet infection and does not persist for long in the environment. Clinical signs and nasal shedding of the virus can occur within 24 hours of infection, and shedding typically ceases by 7 - 10 days after infection.

The course and severity of influenza virus infection in a pig is influenced by management, intercurrent infections, age, immunity and virus subtype involved. The clinical signs of influenza in pigs are remarkably similar to the symptoms in humans, and are typically characterised by an abrupt onset and short course of fever, anorexia, lethargy, coughing, dyspnoea, and nasal and ocular

discharge. Some pigs show loss of condition but recovery usually occurs suddenly at 5-7 days after the onset. Morbidity in non-immune herds tends to be high (approaching 100%) and mortality low (1-4%). In some herds infection may be completely subclinical and can only be detected by seroconversion. Outbreaks may end abruptly or continue in finishing pigs with new cases for several months. The severity of swine influenza infection may be much increased by concurrent infections with other viral or bacterial pathogens resulting in porcine respiratory disease complex.

Infection is generally limited to the respiratory tract with specific tropism for bronchial and bronchiolar epithelial cells within a few hours after infection. Infection of alveolar septae and ducts occurs at the same time. The microscopic lesions develop as congestion followed by focal necrosis of bronchial and alveolar epithelium, with sloughing of affected cells. Finally, interstitial pneumonia and hyperplasia of the bronchial epithelium also occur. Macroscopically the cranioventral lungs are most consistently affected and show sharply demarcated dark red or purple areas of consolidation.

Although the epidemiology, clinical signs and pathology may all be highly suggestive of influenza, a definite diagnosis is possible only in the laboratory via virus isolation, detection of viral antigen or nucleic acid, or demonstration of virus-specific antibodies. The specimen of choice for virus isolation from a live animal is nasal or pharyngeal mucous obtained by swabbing the nasal passages or pharynx. Trachea or lung tissue should be collected in dead pigs.

Biosecurity and vaccination are the primary preventative measures. Prevention of contact between susceptible and infected animals is important but because interspecies transmission is also possible, measures aimed at preventing contact with avian species and humans suspected of having influenza should also be instituted. Several commercial inactivated and adjuvanted vaccines are available both in Europe and USA. The vaccine strain composition differs between continents because of the antigenic and genetic differences between swine influenza viruses circulating in Europe and USA. A vaccine against pH1N1 has also recently become available in the USA[18,23].

SWINE INFLUENZA IN SOUTH AFRICA

Kenya is the only country on the African continent that has reported swine influenza[21]. Recent outbreaks in South Africa of Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS) were successfully controlled and the country is now free of these as well as other economically important virus diseases such as Swine Influenza, Foot-and-Mouth Disease and Pseudorabies (Aujeszky's Disease).

The South African Pig Producers Organisation (SAPPO) in conjunction with the Pig Veterinary Society (PVS) of the South African Veterinary Association (SAVA) conducts regular serological surveys of the country's pigs to confirm freedom from certain diseases, including the virus diseases mentioned above. Serological surveys conducted in 2000, 2004 and 2007 confirmed freedom from swine influenza virus (H1N1) in the national commercial South African pig herd[8]. For the 2009 survey, again all serum samples tested negative for antibodies against swine influenza virus (H1N1) (P Evans, SAPPO, pers. comm., 2010).

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A SYSTEM FOR CONTROLLING GALLEY WASTE DISPOSAL IN GAUTENG

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ABSTRACT

Galley waste is defined as that food waste which emanates from the kitchen (or galley as it is known) of planes and ships. This food, which can emanate from countries where Foot and Mouth and other animal diseases are present, may spread diseases. The disposal of the galley waste should therefore be controlled.

In South Africa, the Foot and Mouth outbreak in Kwazulu Natal and the Classical Swine Fever outbreak in the Eastern Cape are examples where galley waste have been implicated in animal disease outbreaks. Pigs are particularly susceptible to these outbreaks as it is a common practice in this country to feed swill. When there is a lack of control over galley waste, swill from planes and ships may be fed to pigs resulting in outbreaks of some highly infectious diseases with disastrous consequences for export and for the entire countries' animal health. In countries like Australia and New Zealand, galley waste is incinerated but some other countries have regulations that allow the use of landfill. This system is more applicable to the South African situation.

Gauteng Veterinary Services has long recognized that there is a risk associated with galley waste that needs to be managed and controlled. While the Department of Agriculture, Forestry's and Fisheries has a responsibility at the ports of entry, the waste leaves the port of entry and is disposed of in the province. Thus there is a need for a joint national and provincial solution. The Animal Diseases Act grants wide ranging powers to the state to control disease. Nonetheless, it has been difficult to control galley waste directly without an adequate legal mandate. The use of import permits to control the handling and disposal of international galley waste would give the required legal mandate for the inspection and control of the galley waste at the kitchens accepting such waste from international flights. As the kitchens are situated in the provinces, these inspections are done by the provincial veterinary services. Waste depots are registered for the receipt of international waste and these too are inspected by the veterinary services. The implementation of this system has been rolled out at OR Tambo International Airport (ORTIA) in preparation for 2010.

We wish to report on this system as well as an adaptation of the system employed at Lanseria International Airport and the successes and challenges we have had.

INTRODUCTION

Animals and animal products have long been shown to transmit diseases both to other animals and humans. The Foot and Mouth outbreak in Camperdown and the African swine fever outbreak in recent years were both thought to be caused by the feeding of food waste (also called galley waste) from ships that offloaded the waste which was subsequently fed to pigs. In terms of risk, there is a distinction between the galley waste emanating from harbours and airports and it is recognized that the galley waste from ships being of greater volume carries a greater risk. Never the less, galley waste associated with airports does have some risk and must be controlled.

In many countries (Australia[1] and New Zealand[2] for example), galley and cabin waste as well as all agricultural produce is disposed of by incineration by the inspection services. Other countries like Canada[3] for example use a controlled disposal system which utilizes approved landfill.

The major risk situation which we need to avoid is the feeding of confiscated product and galley waste to pigs as swill. The feeding of swill is a common practice in South Africa and one that is associated with major disease risks. Pigs can be infected with the following diseases when feeding swill: Foot and Mouth, African and Classical Swine fever, Porcine reproductive and respiratory syndrome (PPRS), H1N1, Salmonella and Campylobacter. Since they often excrete large amounts of virus, they in some cases then can infect other animals and cause major outbreaks.

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THE CURRENT SITUATION IN GAUTENG

OR TAMBO INTERNATIONAL AIRPORT

CONFISCATED AGRICULTURAL WASTE

The confiscation of agricultural products and the control of agricultural products coming from ships and planes is a national competency. In the past, the focus has been on the confiscated food waste and the issue of galley waste from planes had largely been ignored.

Confiscated waste consists of the unpermitted agricultural products that passengers bring in as well as any food product that is sent into the country via the air parcel post. Confiscated waste at ORTIA has been incinerated at the incinerator at ORTIA quarantine station. The process of confiscation has been further improved by the use of sniffer dogs and the quantity of agricultural waste being confiscated at ORTIA by APHIS (Animal Plant Health Inspection Services) has increased enormously. After the initial success, APHIS purchased an additional number of dogs. These are a common sight in the passenger areas. The amount of waste confiscated has increased.

There are still challenges in the procurement of diesel for the incinerator and currently the confiscated waste is being deposited in a covered tip until diesel becomes available for it to be incinerated. This has some risks attached to it as the waste attracts flies and other vermin. In the long term, it might be necessary to explore other options for the disposal of this type of waste as incineration is not a green option.

GALLEY WASTE

This is the unused kitchen waste and left over food from the flights. At present, it is collected by the kitchens from the plane in closed trucks and taken to the international kitchens adjacent to the airport for disposal. The cabin waste which is the waste left by passengers in the cabin can also contain small amounts of food waste and this is collected by the cleaning service and sorted on the apron. The various pathways by which waste leaves the plane are shown in Figure 1.

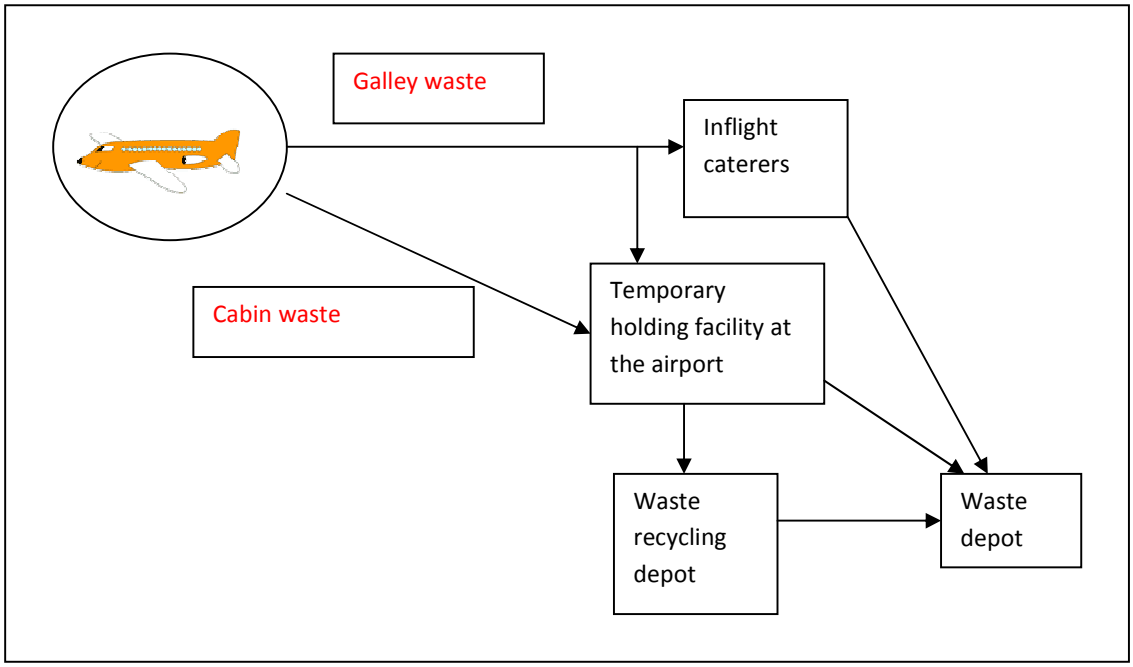

Figure 1: The various pathways by which waste leaves the plane.

Because of the complexity of the waste situation, the disposal of this waste has largely been left to the kitchens and in the case of charter planes, even the individual. Various studies done previously have shown that this has been fed to pigs, and this is cause for grave concern. There was clearly a need for this to be controlled. After much thought, a system of issuing of permits was introduced. Thus in the same way as any animal or animal product entering South Africa requires a permit, so food waste entering the country also requires an import permit. Like any other permit, the import of food waste is subject to certain conditions. These conditions relate to the storage and disposal of the waste in a safe and auditable fashion.

There are currently 3 main International Kitchens at ORTIA and these have been very cooperative. Gauteng Department of Agriculture and Rural Development (GDARD) has identified a waste depot (Rooikraal) with sufficient security and procedures in place to be able to accommodate international waste and in future all waste will be sent here for a safe disposal (a type of disposal that involves the immediate burial of the waste and prevents any access to it by scavengers). It was decided not to use the site for hazardous waste disposal as it is expensive, with limited space and probably not necessary. It is envisaged that APHIS will eventually negotiate with the Airports Company and request that only kitchens that have permits for handling international waste will be permitted to operate on the apron of the airport. Furthermore, GDARD will issue permits to all the kitchen trucks that are carrying kitchen waste that can be checked at the exits to prevent illegal waste from even exiting the airport.

Once this system is well established, with continued inspections of the kitchen and waste depots, there should be adequate control over the food waste. As with Lanseria, there are a number of private planes that also land and generate very small but potentially hazardous waste. This issue has yet to be addressed at ORTIA.

LANSERIA AIRPORT

Lanseria is a small privately run international airport. It has a few large international flights and many chartered flights. These small planes ferry the owners or a few people from all over Africa into South Africa. There is often no identified catering company and food is often brought into the country as the owners exit the apron in vehicles, having cleared customs. APHIS does have a presence at the airport but with only small amounts of agricultural produce being confiscated, holding space is a problem and the confiscated produce has always ended up in the normal waste stream in the past. There are some small kitchens that also utilize the ordinary waste streams. These kitchens are occasionally asked by their clients to hold food items and flowers originating from overseas until they can be used for the next flight out. It is a common occurrence that these items get disposed of by the kitchen in the normal waste stream.

It is clear that permitting the kitchens which seem to work well as a solution for ORTIA is not a viable option for Lanseria. The reason for this is that there are numerous small flights and these can be catered for informally. The amount of waste per flight is generally very small. We assessed the waste site that is currently being used at Lanseria and were not happy about the security, the presence of birds and lack of rodent and fly control, the presence of scavengers and general tidiness. Lanseria is in the process of building a new waste facility that is situated away from the airport and GDARD will have some input in its design and hopefully the issue of international waste disposal will be solved in a more sustainable way.

For now, it was decided that the galley waste removal can be linked to the APHIS inspection and all international flights should hand in galley waste at the customs clearance. Furthermore, the planes must be inspected for galley waste and this can be done in conjunction with the Department of Health who go out to the planes for spraying them. This waste will be preserved and rendered inedible by the use of a 5% formalin coloured spray, stored in a locked area and then taken by GDARD across for incineration to the ORTIA quarantine station. The airport authority is being issued with a permit to import galley waste and make arrangements for the storage and disposal of the waste.

There has been a real attempt to raise awareness of the waste issue at Lanseria by emailing pamphlets to all the tenants. We are not at all sure that there is going to be compliance as there appears to be many ways of exiting the airport and security may not be as tight as it should be. We expect to have challenges to solve as soon as we start to work there and see the constraints that there are.

WATERKLOOF AIRPORT

Waterkloof airport is the military airport and also receives international travellers and must therefore also have international waste issues. We cannot easily gain access there and therefore have no knowledge of the waste disposal that they use. This is something that must be investigated in the future.

SUMMARY

There are 2 different ways of controlling Galley waste in Gauteng. In the case of large international airports, control is mainly through the kitchens that service international flights and making sure that disposal happens at a controlled dumping site. (A controlled dumping site in this context is one that is registered and inspected by veterinary services for the purposes of accepting international waste). In the case of smaller international airports, the control of galley waste is much more difficult and carries a higher risk. Galley waste needs to be controlled within the confines of the airport and this waste can either be incinerated or disposed of at a controlled landfill as described above. The airport authority in this case is issued with a permit to import galley waste.

Since GDARD officials are assisting the Lanseria APHIS personnel during 2010, there is an opportunity to test the system and make further recommendations to solve this potentially risky problem.

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3. www.inspection.gc.ca › Animals › Imports › Policies / Procedures Canadian Food Agency Guidelines for the Handling, Removal, and Disposition of International Waste at Airport Terminals and Sea Ports

RESURGENCE OF FOOT AND MOUTH DISEASE IN THE SOUTHERN AFRICA DEVELOPMENT COMMUNITY (SADC) REGION – CHALLENGES TO THE CONTROL STRATEGIES

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SUMMARY

After a period of over 20 years with isolated low incidence of foot and mouth disease (FMD) outbreaks in the region, the period between 2002 and 2009 saw a resurgence of FMD in the region.Outbreaks of the disease were recorded in Botswana, Malawi, Mozambique, Namibia, South Africa, Zambia and Zimbabwe. The increased disease spread was not only threatening exports to the lucrative European Union (EU) markets that some of these countries enjoy but also presented a challenge on the national control strategies including vaccinations that had proven to be effective for many years in the past. This paper discusses the resurgence of FMD outbreaks in the region, discusses extensively the epidemiology of the outbreaks and examines the challenges posed on the FMD control strategies. The current control strategies are reviewed, and recommendations and suggestions are made on how to strengthen them.

INTRODUCTION

Foot and mouth disease Southern Africa is unique in the sense that the buffaloes (*Syncerus caffer*) are ancient carriers of the Southern African Teritories (SAT) viruses. This adds to the difficulty and complexity in controlling FMD. This difficulty is further magnified by the fact that most countries in the Southern Development Community (SADC) are not involved in intercontinental trade of livestock and pastoral as well as communal farming systems predominate in such countries. There has also been a considerable increase in wildlife based activities like game farming and wildlife conservations competing in most cases for the same grazing land (Vosloo W., Bastos A.D.S., Sangare O., Hargreaves S.K. & Thompson G.R. (2002)).

Despite these challenges, some countries in the SADC region have successfully controlled the disease to ensure access to the lucrative international markets. This success has been achieved by segregation of domestic livestock from buffaloes by construction of buffalo fences and wildlife conservation fences, movement control which depends on adequate fencing infrastructure, permit system and regulations. Early detection of disease through active surveillance as well as vaccinating at least twice a year where buffaloes are present has also contributed to the success of these control measures. This has allowed these countries to be recognized by the World Organization for Animal Health (OIE) to have FMD free with vaccination and FMD free without vaccination zones (Vosloo et al 2002).

However after a period of over 20 years with isolated low incidence of FMD outbreaks in the region, the period between 2002 and 2009 saw a resurgence of the disease in the region. Foot and mouth disease outbreaks were recorded in Botswana, Malawi, Mozambique, Namibia, South Africa, Zambia and Zimbabwe (Baipoledi, E.K., Matlho, G., Letshwenyo, M., Chimbombi, M., Adom, E.K., Raborokgwe, M.V. & Hyera, J.M.K. (2004); Letshwenyo, M., Mapitse, N. & Hyera, J.M.K. (2006)). The increased disease spread was not only threatening exports to the lucrative EU markets that some of these countries enjoy but also presented a challenge on the national control strategies including vaccinations that had proven to be effective for many years in the past. In the following sections, an account of the FMD outbreaks that re-occurred within the SADC region during the 2002 – 2009 periods is given. The outbreaks are discussed in relation to various issues including the possible points of origin as estimated by molecular epidemiology studies, control and challenges.

RESURGENCE OF FMD IN SADC REGION

DESCRIPTION OF OUTBREAKS

The FMD outbreaks that occurred in the SADC countries during 2002 – 2009 periods are summarized in Table 1. Figure 1 presents the geographical dispersion of these outbreaks. In 2002 and 2003 Botswana experienced FMD outbreaks in FMD free without

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vaccination zones along the border with Zimbabwe after a period of about 20 years without outbreaks. These outbreaks were linked to the outbreaks in Zimbabwe at the time and the socioeconomic situation resulting from the political situation in Zimbabwe at the time (Baipoledi et. al., 2004). However from 2005 to 2009 Botswana started getting outbreaks in FMD free with vaccination zones. The outbreak of 2007 started in an area around the Okavango delta preceded by drought followed by floods and over flooding of the delta resulting in fence destruction and mixing of buffalo with cattle. This was also the case with the outbreaks in Malawi, Namibia and Zambia from 2007. These outbreaks were all characterized by spread until 2009 along the normal trek routes of animals as they move from dry to wet areas before the rainy season and back after the rainy season. The outbreaks were also characterized by low prevalence, less than 2.5% in all the counties - probably following crush pens with low vaccination history and coverage and incidence of cases increasing with increase in time from last vaccination.

MOLECULAR EPIDEMIOLOGY

The SAT 2 outbreaks of 2007 lasted until 2009 and brought a lot of questions relating to the relevance of vaccines. These viruses did not change a lot over the period of the outbreaks, only 3%, but the molecular difference between the vaccine strain used and these viruses was ranging from 22 to 24%. Figure 2 shows the molecular relationships between the virus types isolated from various SADC countries. The virus isolated from Namibia and Zambia outbreaks was different from the virus isolated from Botswana. It is worth noting that when vaccination was done according to the manufacturer's instruction, which is *primo* vaccination followed by booster, together with improvement of coverage the outbreaks were successfully controlled indicating that the vaccine successfully reduced virus transmission (Figure 3) (Orsel K., Dekker A., Bouma A., Stegeman J.A. & de Jong M.C.M. (2005); Orsel K., de Jong M.C.M., Bouma A., Stegeman J.A. & Dekker A., (2007)).

DISCUSSION

In the middle of this past decade global warming effects finally hit the SADC region resulting in changes in the normal predictable weather condition. The region experienced rain during the winter seasons, flooding year after year which resulted in the destruction of infrastructure like fences and movement of wild animals out of normal conservation zones. It appears that the region was not ready to factor in these effects in the normal control measures. Perhaps there has also been too much reliance on vaccination only which will fail however if the virus challenge is increased over a period of time (Kitching R.P. (2002)). Vaccination strategy needs to be enhanced in order to improve herd immunity by adhering to vaccine manufacturer's recommendations of vaccine handling and vaccination protocol as well as improving vaccination coverage. Quality assurance, auditing, monitoring and evaluation techniques need to be introduced to assess the effectiveness of vaccination strategies. It is also vital to reassess the risk period and target vaccination in such a way that herd immunity is highest at this time.

The surveillance methods used are based on detecting disease presence. This means that the virus is allowed to circulate until such a time the animals develop lesions. In vaccinated herds the virus may circulate in a lot of animals before the disease is seen (Kitching R.P. (2002)). With development of appropriate diagnostic techniques, particularly tests aimed at detecting the non-structural protein (NSP) component of FMD virus, it will therefore be valuable to institute surveillance methods aimed at detecting presence of virus in order to institute control measures even before the disease is seen (Paton D.J., de Clercq K., Greiner M., Dekker A., Brocchi E., Bergman I., Sammin D.J., Gubbins M. & Parida S. (2006).).

Other elements which need to be factored in the control strategy are the socioeconomic issues affecting the farmers in the remote areas where outbreaks of the disease have been recorded. Last but not least, the importance of doing virus surveillance in buffalo to evaluate the relevance of vaccine used and to detect changes in the virus cannot be overemphasized.

CONCLUSION

The resurgence of FMD in the region presents a challenge to the control strategies. These control strategies have to be refined to cope with the new challenges and make them relevant. The production of purified vaccines not only offers the region an opportunity to access the markets but also an opportunity to revise the use of vaccines. Perhaps the use of normal $3PD_{50}$ vaccine for prophylaxis which results approximately with 80% of animals having protective antibodies extrapolating data from published studies (Ahl, 1990) from needs to be revised and the region should recommend the use of $6PD_{50}$ for prophylaxis which results in more animals having protective antibodies following vaccination and probably compensates for low coverage and farmer attitudes towards vaccination and a $10PD_{50}$ vaccine be used to control disease during outbreaks as practiced all over the world.

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Ahl (1990) Relationship between Serum Neutralising Antibody Titre (Log SN), and Potency According to PD50 Value and Percentage Protection

Table 1: Types of FMD recorded outbreaks in the SADC states since 2002

	BOTSWANA	MALAWI	NAMIBIA	ZAMBIA	ZIMBABWE
2002	SAT 2				SAT 2
2003	SAT 1				SAT 1
2004				SAT 1	
2005	SAT 2				
2006	SAT 2 and SAT 1				
2007	SAT 2	SAT 2	SAT 2	SAT 2	
2008	SAT 2	SAT 2	SAT 2	SAT 2	
2009	SAT 2	SAT 2	SAT 2	SAT 2	

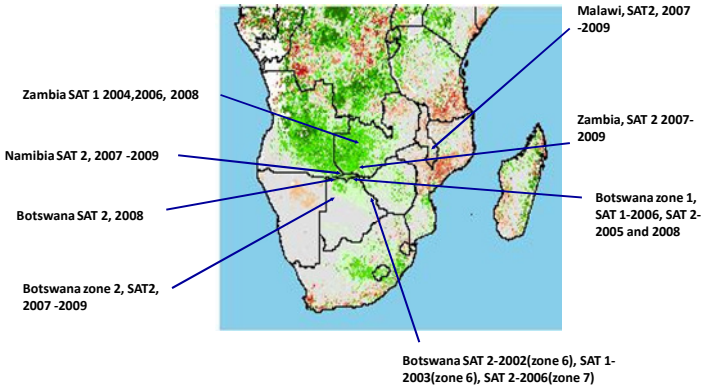


Figure 1: SADC FMD situation 2002-2009

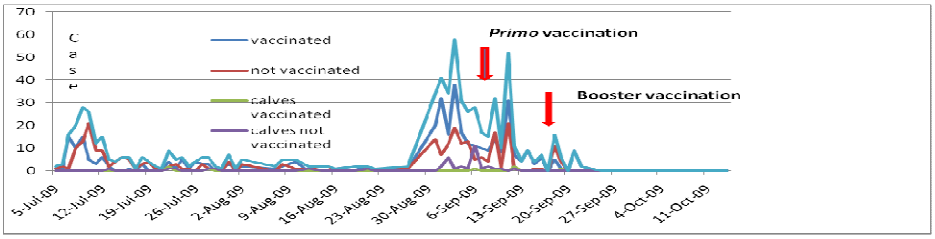
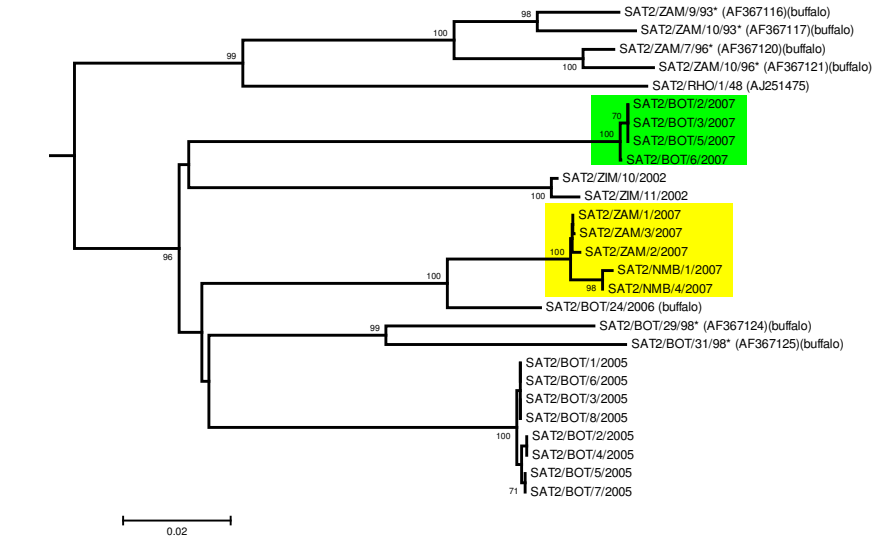


Figure 2: Typical epidemic from the outbreaks showing upsurge of cases 4 months after vaccination and the disease spreading to another area to cause a bigger outbreak 6 months after vaccination. A primo vaccination followed by booster vaccination controlled the disease completely within a month



Mid-point-rooted Neighbor-joining tree (Kimura 2-parameter model) constructed using MEGA 3.1 based on complete VP1 nt sequences.

Figure 3: Relationship between outbreaks of FMDV type SAT 2 in Botswana, Namibia and Zambia in 2007

POSSIBLE EVIDENCE FOR SILENT CIRCULATION OF A SAT-3 FMD VIRUS IN CATTLE POPULATIONS ADJACENT TO GONAREZHOU NATIONAL PARK, ZIMBABWE.

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SUMMARY

Foot-and-mouth disease (FMD) is a highly contagious viral disease and one of the most economically important cattle diseases in Africa. Its endemicity in Southern African countries is mainly due to the presence of large populations of FMD infected African buffaloes (*Syncerus caffer caffer*), reported to be the major maintenance wildlife hosts of the SAT serotypes of FMD virus occasionally transmitted to domestic cloven-hoofed animals. The Great Limpopo Transfrontier Conservation Area connects conservation areas across Zimbabwe, Mozambique and South Africa. Due to the facilitated mobility of wildlife it is anticipated that the risk of spill-over infection of new FMD serotypes and intratypic variants from wildlife to surrounding communal cattle will increase. To assess the efficacy of the vaccine currently being used in Southern Africa, field studies were conducted in a high risk area for FMD virus circulation at the periphery of Gonarezhou National Park in Zimbabwe. Two cattle areas were selected according to the extent of the wildlife/livestock interface and the associated low or high risk of contacts with wildlife. In each area, one diptank was vaccinated with a commercially available trivalent FMD vaccine and another one left unvaccinated and used as a control population. Animals in vaccinated diptanks received a first dose of the vaccine at T0. In order to test the effect of a second primary vaccination, half of the animals received a second dose at T0+28 days. All the vaccinated animals received a third dose at T0+180 days. Antibody titers were monitored at set intervals over a 12 month period. A Non Structural Protein (NSP) test was used on a subset of samples to differentiate between viral and vaccine antibodies and confirm infection. Antibody responses, particularly to SAT-3, in a large proportion of unvaccinated animals and the presence of NSP antibodies in both vaccinated and unvaccinated animals suggest that an outbreak of FMD may have occurred during the study period in the monitored diptanks. However, no clinical signs were detected or reported during the course of the study. These findings raise the possibility of the circulation of very mild or silent FMD virus strains among cattle adjacent to Gonarezhou National Park. However, additional analyses are required to confirm this hypothesis.

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RABIES IN THE EASTERN CAPE IN SOUTH AFRICA: PRELIMINARY STUDIES ON THE CHARACTERISTICS OF THE DOG POPULATION, VACCINATION CAMPAIGN AND ANTIBODY RESPONSE IN DOGS*

S.J. Van Sittert[1], G.W. Akol[1], B. Mlahlwa[1], J. Raath[2]; J.M. Miyen[3] & C.T. Sabeta[3]

INTRODUCTION

Rabies is a growing problem in the Eastern Cape Province of South Africa, with more human cases reported during 2008 and 2009 than in any other province (Blumberg *et al.* (2010)). This abstract outlines preliminary findings of a study aimed at identifying some of the key ecological factors that seem to influence the successful immunization of dogs against rabies in a communal farming area in the Eastern Cape. Factors that may influence the ability of dogs to respond effectively to rabies vaccines were also investigated.

MATERIALS AND METHODS

The Emalahleni Local Municipality was used as study area. It comprises an area of 3550.99 km^2 and was formerly part of the western Transkei. The central town, Lady Frere, is located at 27°14'E; 31°42'S. Weighted/biased random sampling using ArcGIS® software (ESRI Ltd) was used to collect data from 203 dogs throughout the district. Data regarding dog ecology were obtained through owner interviews and serum rabies neutralising antibody titres were determined through FAVN tests. All the dogs were vaccinated with 1 ml inactivated rabies vaccine subcutaneously (Rabdomun® (Intervet/ Schering-Plough (Pty) Ltd). Serum rabies neutralising antibody titres were determined 30-60 days later from eighty randomly selected dogs.

RESULTS AND DISCUSSION

The population was remarkably similar in size, breed, and condition. Slightly over a fifth of the population was between 6 weeks and 1 year of age, while 1% of dogs were 10 years or older. Animal Health Technicians (AHT's) achieved vaccination coverage of 65% of owned dogs over several years, but only 56% of the dogs were vaccinated within the previous 12 months. Merely 32% of the dogs sampled had adequate circulating rabies virus neutralisation antibodies (≥0.5IU/ml). After vaccination 83% of the dogs had seroconverted to this level. The magnitude of seroconversion was independent of body condition, age or previous vaccination status. A new strategy on rabies vaccination is proposed whereby AHT's are pooled together, moving from village to village throughout the district, as opposed to individual AHT's serving only their area of responsibility. A preliminary campaign has found this approach to increase vaccination figures to 72% of the owned dog population, but it also led to an increase in the total time spend on the campaign as well as an increase in transport costs.

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THE CONTROL OF RABIES IN SOUTH AFRICA: WHAT WENT WRONG? (A need for a Holistic, Collaborative and Participatory approach)

I. Lwanga-Iga[1] & S.H Kroll-Lwanga-Iga

ABSTRACT

Rabies Control Worldwide is still a major challenge and South Africa is no exception in spite of over two decades of intensive efforts locally. "Every ten minutes somebody dies of Rabies worldwide" between 24,000 and 70,000 human deaths are recorded in Africa alone (Zinsstag et al., 2008) and over 3 billion people are exposed to Rabies World wide (WHO, 2005)

The classic strategies of rabies control commonly practiced worldwide lie mainly in the following areas namely, canine population management, mass vaccinations, concerted surveillance and lastly education and mass mobilization awareness programs (WHO, 2005). Most of the above have also been applied in various provinces in South Africa to a varying extent. In agreement with many other authorities on Rabies control Lembo et al. (2010) believe that the global challenges in Rabies control are mainly caused by the fact that this disease is regarded as a low priority in developing countries coupled with epidemiological constraints, operational constraints as well as limited resources.

The uniqueness of South Africa lies in our social strata, the magic of having the super rich living side by side with the abject poor, the educated with the uneducated, the third world directly interfacing with the first world on one plate, the subsistence sharing the same market with the commercial, the informal sharing the same space with the formal. Understanding our social challenges and using them positively to contribute towards what we need to achieve is the way to go. The current practice of "cutting and pasting" and importing of non-customised so called "Best Practices" will unfortunately not work. Purely "Formula and Rule based" control strategies will have to give way to those which put the community and people at the centre of rabies control in a participatory manner.

The main thrust of rabies control in South Africa should be directed towards the peri-urban and rural areas, that is where the main problem lies. These areas need a purely "home grown South African solution."

This paper proposes that all things being equal, the solution lies in holistic, collaborative, people centred approaches which would involve mass mobilization, participation of the communities, improved community education and looking beyond ourselves.

KEY WORDS

Rabies Control, Public participation, Holistic, Collaborative

INTRODUCTION

The control of rabies worldwide is still a major challenge and South Africa is no exception in spite of over two decades of intensive efforts locally. "Every ten minutes somebody dies of rabies worldwide", between 24,000 and 70,000 human deaths are recorded in Africa alone (Zinsstag et al., 2008) and over 3 billion people are exposed to rabies worldwide (WHO, 2005)
In spite of concerted efforts both locally and internationally the disease continues to be viewed as a serious public health challenge to which we have no solution to. Various workers in this area both in practice as well as in academia have raised various concerns especially as regards to the social scientific aspects of this disease (Nel et al 2009; Gummow et al 2010) There are also very specific studies and reports in the SEARG Proceedings over the years about rabies in South Africa as far back as 1993 which bring similar concerns to the fore (Akol et al 1993, Randles 2003, Perret 2005). These findings and recommendations seem to be falling on blind ears in South Africa since to us as natural scientists this is an "alien" field which we neither engage in during our training nor important to us as implementers: thus an area best left to the "politicians and social academics". But it is an area all epidemiologists must delve in.

The purpose of this paper is to provoke thought and categorically state that rabies cannot be controlled in South Africa unless certain critical elements from social science studies and reports are integrated into our control approaches and probably paramount here would be to initiate the so called "people centered approaches". Whereas many scientific papers mention these, they do fall short in getting deeper into the individual elements they are referring to (Gummow et al 2010).

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BACKGROUND

Every ten minutes somebody dies of Rabies somewhere in the world and chances are that one of those could be our own here in South Africa, and in most cases it is likely to be a child. Rabies cases are increasing both in animals and humans in South Africa especially in KZN, Eastern Cape and Limpopo.
A very interesting study by Cohen from Limpopo found that there were 5 animal cases in 2004 which progressively increased to 100 in 2006. The human picture in the same study found out that 21 confirmed, 4 probable and 5 possible cases were recorded from August 2005 to December 2006 (Cohen. et al 2007). The picture in Eastern Cape is not any better where both the animal and human cases have been on the rise in spite of the various control efforts currently in place. With reliable reporting I am convinced that the trend would not be any different in other areas of our Republic. We are losing the battle to control Rabies in South Africa.

Rabies control in South Africa over the last few decades shows a situation which is not improving. The following two maps show that the trend has remained more or less static in that there seems to be neither marked change in the areas affected nor a major reduction in the number of outbreaks. This picture is also mirrored in the human cases as reported in various National and Provincial quarterly and other special reports.

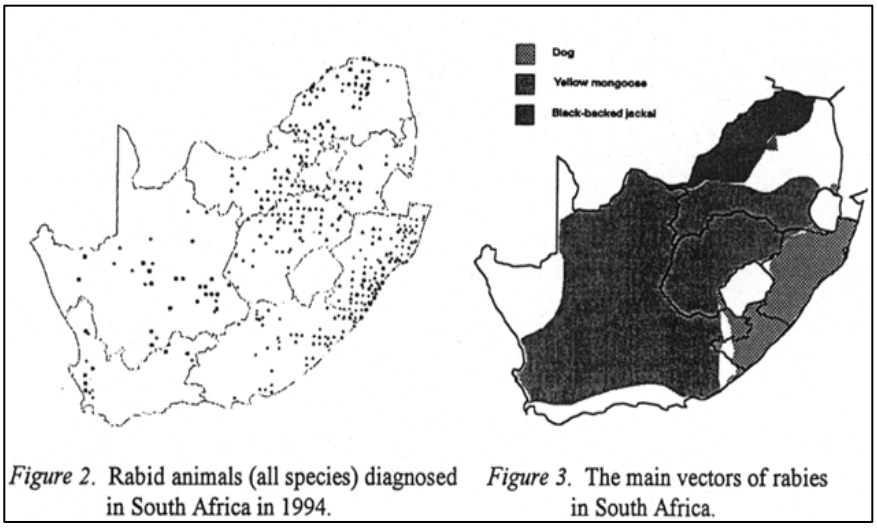


Figure 2. Rabid animals (all species) diagnosed in South Africa in 1994.

Figure 3. The main vectors of rabies in South Africa.

Figure 1: The above map shows the animal rabies outbreaks in a study by Bishop in 1995

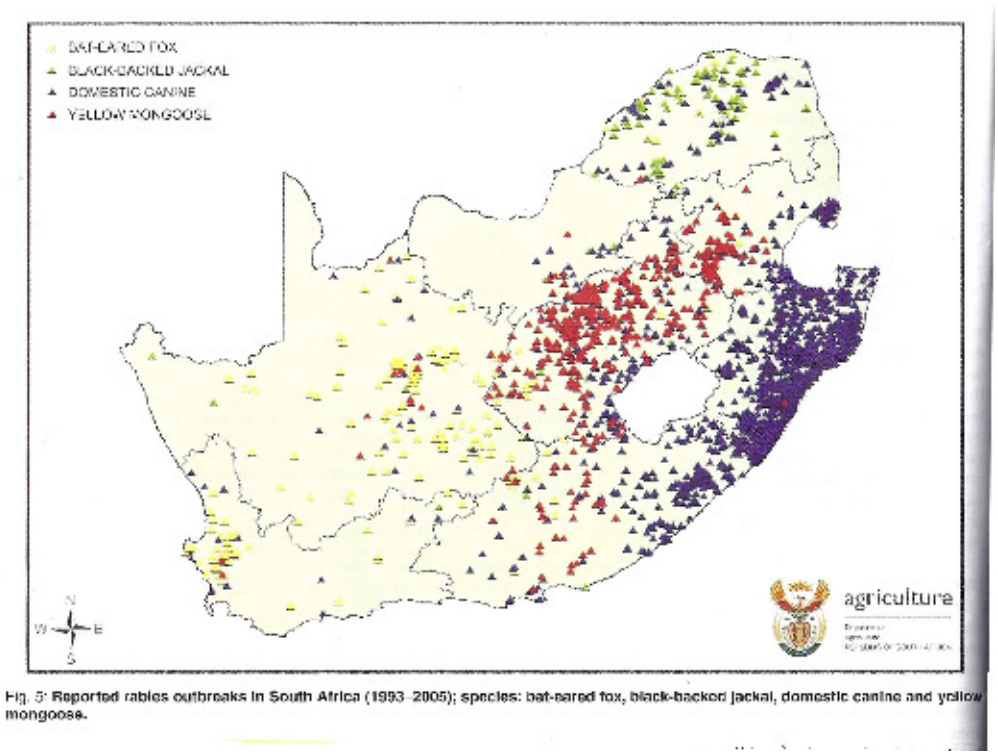


Fig. 5: Reported rabies outbreaks in South Africa (1993–2005); species: bat-eared fox, black-backed jackal, domestic canine and yellow mongoose.

Figure 23: The above map shows the rabies outbreaks in a study covering 10 years later in 2005 (Gummow et al 2010)

ZOONOTIC DISEASES CONTROL STRATEGIES

The successful control of Zoonotic diseases according to the FAO-OIE-WHO tripartite is anchored mainly in four areas namely:

1. Improved diagnostics
2. Effective epidemiology
a. Reliable data and analysis
3. Communication
4. Aligning activities related to the Animal –Human ecosystems

Whereas efforts in most of our academic and practical activities have gone through the first two areas, though not anywhere near the expected levels, it is evident that very little is being done towards the last two because these are areas in which we are not experts as natural scientists or veterinarians. Again these are also areas which are neglected in our studies at centers of Higher learning; at best it is assumed that these could easily be learned, at a later stage. This is unfortunately not the case. According to the FAO-OIE-WHO all the four above are globally important "...in order to achieve more effective management of zoonotic and other high impact disease in the future" (FAO-OIE-WHO 2010:6).

CURRENT RABIES CONTROL STRATEGIES

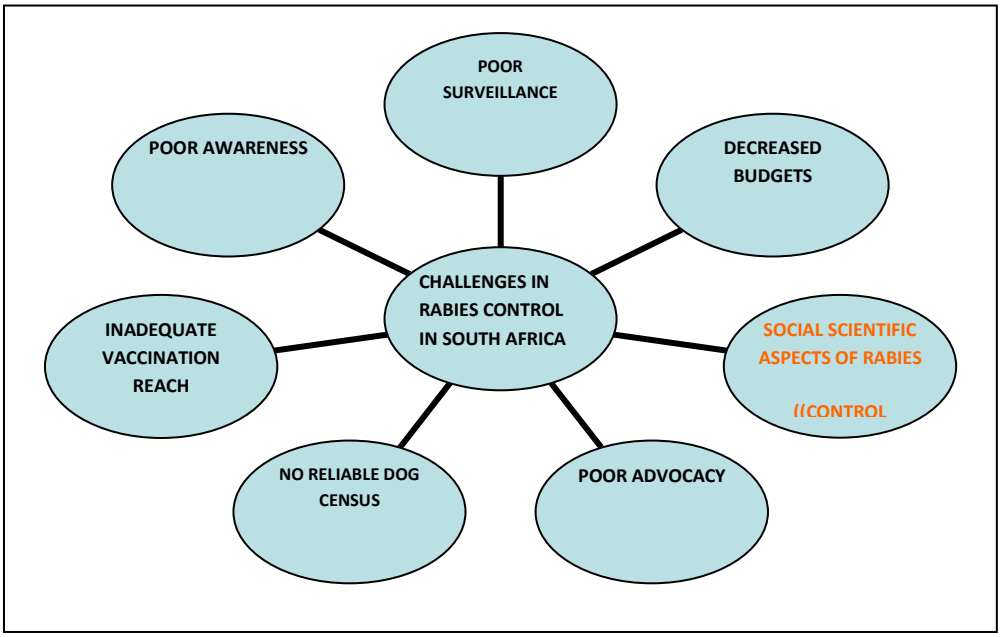
The following are the recognized Rabies Control Strategies and have been used in different parts of South Africa with various degrees of success.

1. Dog Destruction (Strays)
2. Mass Vaccinations
3. Dog population management
3.1 Movement restrictions
3.2 Habitat control
3.3 Reproduction Control
4. National / International cooperation
4.1 Rabies as part of "Key health strategy'
4.2 Medium term planning
5. Rabies Awareness Programmes
5.1 Education / Training

Whereas it is not the purpose of this paper to dwell on the merits or demerits of these strategies, it is important to note that a glaring result of the concurrency governance of veterinary services in the country, the Provincialization process has indeed made it very difficult to have a uniform approach to rabies control and which strategies to put emphasis on. As a result of this, and whereas the National competency might have the overall oversight on controlled diseases, the final word , choice and prerogative lies in the hands of the Provincial competency (Constitution of South Africa Act 108 of 1996)

CURRENT CHALLENGES IN RABIES CONTROL IN SOUTH AFRICA

Various authors and practioners have recognized challenges which are perceived to be the main stumbling blocks in the control of Rabies as follows (Hatch et al 2004, Lembo et al 2010, and Gummow et al 2010)



These challenges mirror those which have also been experienced in other Sub-Saharan African countries and in Asia. It is also important to note that these views were confirmed in the latest Post Conference report of the South and East African Rabies Group meeting held in Botswana in 2008 (SEARG Report 2008).
So in effect there is really nothing new. The question is: Why is there no marked progress being made in the control of rabies in South Africa?

A PROVOCATIVE PROPOSAL

The proposal is that what most authors and practioners refer to as “Sociological and Political issues” are indeed “Social Scientific Aspects” which should be at the centre of any activity involving society or people and these are:

1. Understanding South Africa’s uniqueness
2. Understanding our social milieu across the board
3. Exploiting our resources (human, financial, infrastructure)

In other words the integration of certain key social science imperatives into our natural science dictates without a doubt would enhance our rabies control strategies. In fact, most of the so called stumbling blocks are rooted in the fact that we have decided to disregard **“**these social scientific aspects**”** or at best given them lip service.

THE MISSING LINK

The missing link here is that we have failed to implement those vital “social scientific aspects” which are key cogs in the delivery of any service in any community and these are:

1. Participation
2. Collaboration (Classic)

3. Holistic (New way "One Health")

As regards to participation**,** one is putting the **"**people" at the centre of this activity. The issue here is not about dogs, it is about humans, it is about the control of the disease in humans. Our current activities are unfortunately putting the dog in the centre of the control process and I think this could be a misnomer.

Public participation is a process whereby the people are actively involved in the decision, planning and delivery of services which affect their lives. The current Government in South Africa sees this as "...an open, accountable process through which individuals and groups within a selected community can exchange views and influence decisions which affect their lives" (DPLG 2005:1). Brynard in Bekker (1996:133-134) suggests that another definition could be "….purposeful activities in which people take part in relation to a local authority of which they are legal residents".

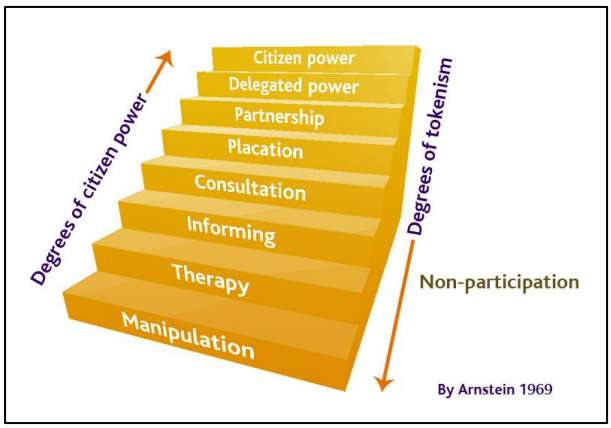


Figure 3: The ladder of Participation (Source: DPLG 2005)

Post 1994 the notion of public participation as a key process in the democratization of governance at the local level is anchored in Chapter 2 of the Constitution, sections 151(1); 152; 195(e); the White Paper on Local government 1999 (Municipalities to develop systems to encourage citizen participation); the Municipal Structures Act 117 of 1998 as amended (Ward council formation , participatory democracy) and the Municipal systems Act 2000 (Communities to work in partnerships with Municipalities, community participation, participatory governance). The need for public participation lies in the following areas namely: consultation as a legal requirement in South Africa, plans and services are tailored to local needs, promotion of community involvement and empowering people to have control over their lives and livelihoods (Craythorne 2006: 263-264).

Participation has been evolving in South Africa over the last two decades. During the apartheid years a system of "community management" did prevail characterized by passivity and dependency, followed by "project based community participation" post 1994 (government expected to deliver services) followed by the "community development approach" (Government acts as gate keeper) culminating in the status quo as of now, namely " Partnerships or negotiated development" whereby the communities participate in the affairs of the municipality knowing their rights and responsibilities. In short, participation allows for policy to be influenced by the people's needs, lives, aspirations and the reality of basic services on the ground.

In view of the above and if indeed the communities are involved in what we are doing from the word go there should be no difficulties in getting the dog census done, making everybody aware, better advocacy and ensuring that there is better funding for these activities and ultimately fulfill the requirements to enable us to control Rabies

Secondly it is also important to look at collaboration**.** The definition of classic collaboration is " Two or more people or organisations working together to achieve a common goal". Collaboration in the classic sense would then involve sharing knowledge, aligning questions, inquiries and building consecus on given issues. The outcomes here would then be learning from others , avoiding duplication , maximizing the use of meager resources in terms of money , infrastructure and specialized personnel and of course greater recognition.
Collaboration in South Africa either internally or externally is proving to be a tall order, not surprisingly because of our past social stand pre 1994 (total isolation) to our current status post 1994 (paste and copying and I know it all attitude). There is still a "lager mentality" in the way we do our business in veterinary services in that we still prefer to work in isolation and with only people we know. Sharing information or activities seems to be a big no no, differing perspectives and views are still a big threat to many, intra and inter provincial cooperation is at its lowest ebb.
Whereas neighbouring countries like Lesotho and Swaziland are reaping the benefits of welcoming various key international partners in the veterinary area, we are still as a country debating whether we should let them in .There is a difference between being a member of the FAO or OIE and to actively making use of the facilities made available by these countries at country level. It is for example an open secret that a lot of programmes being run through the FAO Gaborone Office in SADEC countries are

being hugely propped up by finances from South Africa but unfortunately these programmes are not available here though we have similar needs.

Chapter 3 of the Constitution of South Africa talks about "Cooperate Governance" and the era of the 3 spheres of governance, which brought the Provinces into life. Most of us read this wrong, in fact we put the woes of our failure to control certain diseases in this country to this divide. We still hear calls from various quarters for decentralization of this Service. I would like to call this misplaced optimism and a very retrogressive step to say the least. This view is compounded by those who have failed to read and understand our beautiful Constitution and the various accompanying pieces of legislation in this direction. The problem is that in spite of our world-renowned legislation our problems always start and end at implementation. We are good writers and drafters but very bad implementers.

There are over 15 formal and informal organizations worldwide working on Rabies problems in which we should be having a strong contribution. The reality of this is that we are not represented as a country and therefore it has been left to individuals or provincial initiatives. One glaring example here is that of the current Gates and Melinda Rabies assistance in KZN, whereas similar projects are run in Tanzania and Philippines as a country project, the same is done in South Africa as a provincial project. This project is being run in KZN with probably little involvement from either the National competency or the neighboring provinces. How can you expect such an effort to have any sustainable results? There are also dangers of the donor providing this assistance with strings attached which has been a major problem in the Overseas development assistance milieu over the years (Easterly 2006:5).

Elsewhere the positive spin offs of collaboration have been very well documented, for example, the World Rabies day events happening every year and in which again South Africa does not participate as a country but again it is left to the individual provinces or the recent development of a Rabies Control Blueprint by colleagues sitting in Bamako (www.rabiesblueprint.com).

Thirdly the Holistic Approach (One Health) is now a buzz word which has taken the Public Health community by storm. The Webster dictionary defines Holistic as "Relating or concerned with the whole, or complete systems" (www.merriam-Webster.com). One normally refers to a holistic health programme in humans as looking at the mind, body, spirit and the environment.

Under the One Health umbrella all diseases are looked at in relation to the threat to the triad Man-Animal-Environment, taking the peoples livelihoods as key to the whole process. In this instance a given animal disease is taken as a syndrome which would affect the other components as well In practical teams this calls for "integrated programmes" in Public Health, it calls for integrated planning, it calls for integrated implementation, and it calls for the abolition of the implementation silos. A good example here is found in the Local Government Administration where the production of IDP's (Integrated Development Programmes) is a key requirement in accessing funding (Municipal systems Act 2000).

A holistic approach would then bring about a strong lobby and dedicated advocacy which would assist the Public health system to take the right decisions and also to exploit the symbiotic outcomes of this process (Nel et al 2009). The best route would be to bring Rabies on the National agenda the same way the lobbyists have managed to raise the level of HIV-AIDS to a level where everybody seems to be actively involved in it. The advantages are immense in that Rabies would then be prioritized at a higher level right from the local administration through the Provincial, National and International levels. Because Rabies is a zoonotic disease it would assist in identifying those specific drivers which not only address the Veterinary Public issues but also those which and are not limited to the Socio-economic issues and the Eco-systems. Whereas the former puts the People again at the centre of Rabies Control activities, the latter emphasizes the fact of co-existence between man and animals; brings in the notion of a shared space and the fact that there is that dependency in the form of food and feed, fibre, power and fuel

A very interesting case of a holistic approach in modern times was the discovery of West Nile Disease in the United States of America in the 90's. Whereas West Nile Disease was first seen in Uganda in 1937 and dealt with as an animal disease in East Africa since then, the world was alarmed when through "Holistic approaches" and investigations a colleague discovered that the mystery disease killing the elderly folk in New York in 1999 was indeed West Nile Disease and since then this disease has killed over 1.5 million people in America alone (www.onehealthinitiative.com).

This convergence of Man-Animal-Environment pushes us to see animal diseases like Rabies through a cross-sectorial, multi-institution, and multidisciplinary view whereby the health of one is dependent on the other (www.onehealthinitiative.com). Sustainable efforts to control rabies will have to emerge through such efforts.

CONCLUSION

Rabies control in South Africa can only succeed if there is a correct interplay between our scientific knowledge and the understanding of our local social imperatives as well as the social science "body of knowledge". This fact is well known but has been neglected over time, to our detriment.
The implementation of "One Health is Now**"** because this basically underscores the importance of the 3 social scientific imperatives outlined in this paper.

According to Bernard Vallat, the Director General of OIE:
"Rabies control and eradication requires raising public awareness of rabies and collaboration with all other professions especially in the public health sector." (ARC: 2010).

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THE ROLE OF ZOONOTIC VECTOR BORNE VIRUSES AS NEUROLOGICAL PATHOGENS IN HORSES AND WILDLIFE IN SOUTH AFRICA

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KEY WORDS

West Nile virus, Wesselsbron virus, Sindbis virus, Middelburg virus, Shuni virus, wildlife, horses, rhinoceros and encephalitis

SUMMARY

Mosquito borne viruses in the families -flaviviridea, Togaviridae and bunyaviridae may be the cause of severe neurological disease in humans and animals. These viruses often co-circulate because of similar insect vectors. Although many of these viruses are endemic in Africa, few cases are ever reported and certain members of these families remain uncharacterized as far as pathogenic potential goes. In a recent investigation of the disease potential of West Nile virus in South Africa, endemic lineage 2 strains were identified that may cause fatal neurological disease in horses. Here we report the expansion of this study over 3 years to investigate all 3 viral families in horses as well as wildlife with unexplained neurological signs. This study will contribute to the identification of potential under detected zoonotic viruses and help to determine their contribution to morbidity and death in animals and humans in Southern Africa.

INTRODUCTION

Mosquito borne viruses in the families flaviviridea, Togaviridae and bunyaviridae may be the cause of severe neurological disease and hemorrhagic fevers in humans and animals [1-3]. Although many of these viruses are endemic in Africa, few cases are ever reported and certain members of these families remain uncharacterized as far as pathogenic potential goes. In South Africa, little data is available about the association of endemic arboviruses in neurological disease in humans and animals and most studies were carried out prior to the molecular era [4]. The role of mosquito borne zoonotic arboviruses of these families in neurological disease in horses in Africa is not known. Following the identification of WNV as a cause of neurological disease in horses in South Africa, the question of its importance in annual cases as well as the possible role of co-circulating alpha and bunya viruses were raised. The association of these viruses with unexplained neurological disease in wildlife is also in question.

Mosquito borne Flaviviruses comprise several members that may cause neurological disease in humans and animals. In South Africa the most common flaviviruses are WNV and Wesselsbron virus [4].

West Nile virus (WNV), a mosquito-born flavivirus of the family *Flaviviridae* mainly circulates between birds as vertebrate hosts and ornithophilic mosquitoes as maintenance vectors. Humans and horses are considered incidental dead-end hosts [5]. Approximately 20% of human cases present with fever, rash, arthralgia and myalgia while approximately 1% of these may develop severe disease including meningoencephalitis, encephalitis, polio-like flaccid paralysis, hepatitis, myocarditis, pancreatitis and death [5]. In horses, 20% of cases develop clinical disease of which up to 90% develops neurological disease following WNV infection. Symptoms includes ataxia, weakness, recumbency, muscle fasciculation as well as high death rates (30%) following neurological infections [6]. Genetic lineages include lineage 1, found in North America, the Caribbean, North Africa, Europe and Australia, lineage 2 mainly in Southern Africa and Madagascar [7]; Lineage 3 and 4 in Central and Eastern Europe [8] and lineage 5 in India [9]. Differences in neuroninvasiveness and pathogenic potential is a function of genotype not of lineage [7, 10-12]. We recently reported WNV lineage 2 in several cases of neurological disease in horses in South Africa of which the majority were fatal [11].

Wesselsbron virus is a well known zoonotic veterinary pathogen in South Africa. It is wide spread throughout Sub-Saharan Africa causing febrile illness in sheep and is associated with hepatitis, hemorrhages, abortion and mortality in new-borne lambs. Isolations have also been made from ostriches and a foal in SA[13]. Zoonotic infections have been reported in humans and encephalitis has been reported once [14]. We recently reported two neurological cases in horses one that was fatal (unpublished data).

Alphaviruses in the *Togaviridae* virus family are widespread across the world and are important pathogens for a variety of animals and causes morbidity and mortality in humans [15]. The most common Alphaviruses in South Africa are Sindbis virus and Middelburg virus [16] and are transmitted by *Culex* and *Aedes*[17] mosquitoes respectively. For this reason these viruses are frequently co-circulating with West Nile virus and Wesselsbron virus[18]. Alphaviruses are divided into Old World and New World viruses [19]. Old World viruses are

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mostly associated with febrile, arthritic-type disease in the small joints which may be accompanied by a rash and are rarely fatal although occasional encephalitis cases have been reported in humans; the New World viruses primarily result in severe neurological disease and includes several important horse pathogens such as Western and Eastern equine encephalitis (WEE; EEE) and Venezuelan equine encephalitis (VEE)[19].

The family Bunyaviridae consist of 4 main genera, Orthobunyavirus, Hantavirus, phlebovirus and Nairovirus each that includes several human and animal pathogens. Mosquito transmitted bunyaviruses that may cause disease in humans and animals include Akabane, Oropouche and Shuni virus (orthobunyavirus genus) and Rift Valley fever (Phlebovirus genus). In 2008 we identified Shunivirus in a case of fatal encephalitis in a horse (Van Eeden unpublished data) which lead to inclusion of this virus in the study of causative agents for neurological disease in horses in the country. Shuni virus was first isolated from a cow in an abattoirs in 1966 in Sokoto (Nigeria), from cattle, a sheep and *Culicoides* . A single isolation has also been reported from a child in an outpatient clinic in Nigeria in 1966. In South Africa it has been isolated from mosquitoes, cattle and a goat[20]. In 1977, Shuni virus was isolated from the brain of two horses with severe meningoencephalitis submitted for rabies virus examination; one from South Africa [21] and one from Zimbabwe[20].

Here the combined findings of an investigation of the association of flavi, alpha and bunyaviruses as causes of neurological disease in horses and wild life in South Africa is described over a period of 3 year. Cases of acute disease consisting of mainly neurological disease as well as some cases of fever were screened for flavi, alpha and Shunivirus and analyzed in terms of prevalence, seasonality and symptoms. Phylogenetic analysis was used to characterize the circulating genotypes.

METHODS

Veterinarians were invited to submit EDTA blood or post mortem brain and spinal cord specimens from horses that suffered from acute neurological signs from 2008-2010. Blood and neural tissue from received from horses included 64 samples (37 with neurological symptoms) from 2008; 47(34 neurological) and 83(68 neurological) from 2010. Tissue received from 10 rhinoceros and 2 crocodiles with unexplained neurological symptoms were also included. Family specific RTPCR for flavi-, alpha-, bunyaviruses were used for screening clinical specimens. Realtime PCR for WNV that differentiate between lineage 1 and 2 were used to diagnose WNV cases as described. [22]. IgM ELISA and neutralization tests were conducted for WNV in negative cases that may have passed the viremic stage in 2008 and 2009 but is still in process for 2010. Hemagglutinin inhibition assay (HIA) results were received from Onderstepoort Veterinary Institute for some cases that tested negative by PCR Confirmed cases are indicated as RTPCR positive or IgM AND neutralization assay positive; probable cases were neutralization assay positive with high titres for the virus if IgM ELISA tests were not available (2010). For cases where hemagglutinin inhibition assay results were available with high titres, cases are listed as possible cases.
Differential diagnosis for African horse sickness virus, equine encephalosis and equine herpes virus was also carried out by RTPCR on all positive cases.
DNA sequencing and phylogenetic analysis were used to characterize strains and define genotypes in South Africa.

RESULTS:

In total 194 specimens were received from horses from 2008-2010 of which 139 had neurological symptoms. In 2008 WNV was confirmed in 14 cases and as the probable cause in 7 cases; in 2009 3 confirmed and 12 probable cases were identified while 11 WNV cases were confirmed in 2010. Serological data for 2010 on the other cases is still pending. WNV cases with neurological symptoms included 16 in 2008; 15 in 2009 and 11 in 2010 (16-43% of neurological cases submitted). Of these 7 in 2008, 9 in 2009 and 7 in 2010 were fatal or euthanized (43%-63% of neurological WNV cases). Wesselsbron virus was detected in 2 horses in 2008 with neurological disease, one that was fatal and both with neurological symptoms similar to WNV. Coinfection with African horsesickness virus (AHSV) was detected in 2 WNV cases both with neurological disease, which is atypical for AHSV and that may enhance disease.
For the alpha viruses, in 2008, 2 Sindbis cases were confirmed and 2 possible cases identified. The confirmed cases both only had acute fevers. In 2009 one Middelburg and 4 Sindbis cases were confirmed. The Middelburg case had neurological symptoms but survived while 2 of the 4 Sindbis cases had neurological symptoms and were fatal but had co-infections with WNV. In 2010 nine alphaviruse PCR positive cases were confirmed, all were identified as Middelburg virus (9/83) by sequencing and 4 had neurological signs, (5.8% of neurological cases) the rest had fevers and symptoms similar to three day stiff sickness (arthralgia). Two Middelburg virus cases were fatal.

Electron microscopy identified bunya virus like particles in one virus isolate from a horse with severe and fatal neurological disease in 2009. Subsequent screening of specimens with the bunyavirus family primers confirmed the presence of Shunivirus an uncharacterized virus in the Simbu serogroup, Orthobunyaviridae. Subsequent screening of negative neurological cases with primers specific designed for Shunivirus identified the first 2 cases, in 2009, both with severe neurological symptoms and fatal. In 2010 a total of 4 shunivirus cases (5.8% of neurological cases) were confirmed in horses, all with neurological symptoms, and 2 that were fatal.

Following the identification of flavi, alpha and Shunivirus in fatal neurological cases in horses, neurological tissue from 10 rhinoceros that died with neurological signs were submitted for testing for these virus families, 3 in 2009 and 7 in 2010. Of these 4 were confirmed as alphavirus by RTPCR in the brains in 2010, 3 were shown to be Middelburg virus and 1 Sindbis virus by sequence analysis. One case that was positive for Middelburg virus had a co-infection with Shunivirus. In 2010 brain specimens from two crocodiles with neurological symptoms were also submitted; of which 1 was positive for Shunivirus.
Phylogenetic analysis of the WNV cases confirmed Lineage 2 in 6/6 PCR positive cases in 2008, 3/3 PCR positive cases in 2009 and 5 of 7 PCR positive cases in 2010. In 2 cases identified in 2010 a lineage 1 strain was identified. This was a pregnant mare and her aborted fetus and was detected in Ceres in June. The mare had severe neurological symptoms and died a week after aborting. WNV was detected in the brain of both the mare and foal. This is the first time that lineage 1 has been detected in South Africa and may have been imported by migratory birds from North Africa or Europe.
Phylogenetic analysis of the alphavirus and Shunivirus cases will be discussed in the papers of Human et al. and Van Eeden et al.

DISCUSSION

Using molecular techniques to identify arboviruses have the advantage of increased sensitivity and early detection of viral nucleic acids during acute disease. Since these disease are endemic in South Africa IgG serological tests alone is not sufficient for confirming cases. Nevertheless because of the short viremia and this is likely an underestimate of the true number of cases. Screening for probable cases with IgM and neutralization assays have been carried out of WNV in 2008 and 2009 but is still pending for 2010. Serological tests for Sindbis and Shuni are not yet available. WNV positive cases were identified in Gauteng, the Karoo, North West Providence, Natal, the Freestate; Wesselbron virus in the Western Cape and North West Province; Sindbis virus in Natal, Cape Town and Gauteng; Middelburgvirus in Kwazulu Natal, Swaziland, North West, Gauteng, the Karoo, the Northen Cape, Western Cape and Shunivirus in Gauteng, Northern Cape, Karoo and Limpopo.

All WNV cases were detected between February and July in all 3 years. Sindbis and Middelburg cases were detected between February and June while Shunivirus cases were detected between February and May.
Clinical symptoms for horses with neurological signs were similar between WNV, Middelburg and Shunivirus although a higher percentage of WNV and Shuni cases were fatal compared to Middelburg. Symptoms included ataxia, recumbency, in-coordination, hind and/or forelimb paralysis, muscle fasciculation and seizures in severe cases. Fever was not recorded in all cases. Although some cases that were recumbent and with limb paralysis did recover after a number of weeks several horses died and several were euthanized for humane reasons. No neurological Sindbis virus single infections were detected. The only cases of Sindbis with neurological symptoms were co-infected with WNV.
Rhinos that were positive for Middelburg and Sindbis virus as well as the Middelburg/Shuni co-infection had sudden onset of symptoms, paralysis and sudden death. The crocodile was also paralyzed and died.

CONCLUSIONS

This study confirmed WNV, Wesselsbron virus, Middelburg virus and Shuni virus as neurological pathogens in horses in South Africa while Sindbis appears to be associated only with fevers in horses. In addition detection of Middelburg virus, Sindbis virus and Shunivirus in the brain of rhinoceros as well as a crocodile suggest that these viruses may also affect certain wildlife species. The increase in cases during 2010 may be attributed to both an increase in clinical awareness of neurological disease in horses but also to an unusually high rainfall in the past season and increased mosquito activity. The addition of serological data will likely increase the number of positive cases significantly. This study confirms that horses and wildlife with neurological disease may act as sentinels for vector borne virus activity in Africa and detected both novel pathogens as well as newly imported lineage 1 strains. The value of the animals involved justifies the development of vaccines to protect animals against these viruses.

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MOLECULAR EPIDEMIOLOGY OF SHUNI VIRUS, A CAUSE FOR ENCEPHALITIC DISEASE IN HORSES IN SOUTH AFRICA

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SUMMARY

The cause of many cases of viral encephalitis in humans and animals remains undiagnosed. Several arboviruses with neurological potential such as Flavi-, Bunya- and Alphaviruses are known zoonosis and are therefore of both medical and veterinary importance. Horses in particular are highly sensitive to some of these viruses and have thus been targeted as sentinel animals in the identification of these virus families in neurological disease. Our aim was to identify and characterise unknown viruses in cases of undiagnosed neurological disease that tested negative for WNV and alphaviruses in horses within South Africa. In the process we identified several cases of an uncharacterised bunyavirus named Shuni virus.

INTRODUCTION

More than 300 viruses, mostly arthropod-transmitted, are classified into the family Bunyaviridae, making it one of the largest groupings of animal viruses. The orthobunyaviruses are transmitted by mosquitoes, biting midges and mites, and compose one genus of the family Bunyaviridae. This genus has been subdivided into 19 serogroups, the Simbu serogroup, first described by Casals (1957), is the largest and currently contains 25 related viruses including important pathogens that may cause disease in humans and animals. The majority of Simbu group virus isolations in Africa were made in the course of arbovirus surveys and as such little is known of their veterinary significance.

The prototype virus, Simbu virus was originally isolated in South Africa from pools of *Aedes circumluteolus* mosquitoes caught in 1955 and 1957 at Lake Simbu (Weinbren, Heymann, Kokernot, & Paterson, 1957). Simbu serogroup viruses including Shuni, Sabo and Shamonda have been isolated from apparently healthy cattle, sheep and goats (Da Costa Mendes, 1984). Shuni virus was first isolated in 1966 from a cow in Sokoto (Nigeria), after blood samples were collected from animals at various abattoirs (Causey, Kemp, Causey, & Lee, 1972). It was then again isolated, twice from cattle, once from a sheep (Causey, Kemp, Causey, & Lee, 1972) (Kemp, Causey, Moore, & O'Conner, 1973) and twice from *Culicoides* (Lee, 1979)). Shuni was also isolated from a one and a half year old child at the General Outpatients Clinic at the University College Hospital (UCHGOP) in August 1966 (Moore, et al., 1975). In South Africa it has been isolated from mosquitoes (Coetzer & Erasmus, 1994), cattle and a goat (McIntosh, 1972) (McIntosh, 1980). In 1977, the virus was isolated from the brain of two horses with severe meningoencephalitis submitted for rabies virus examination; one from South Africa (Coetzer & Howell, 1998) and one from Zimbabwe (Coetzer & Erasmus, 1994).
Our study led to the identification of Shuni virus in a horse with suspected viral meningoencephalitis, where viral infection was confirmed by means of tissue culture and electron microscope and the virus then amplified using Bunyavirus specific primers and identified through phylogenetic analysis (Van Eeden, et al., 2009). This finding led to the incorporation of a Shuni virus specific PCR in our diagnostic tests, which has resulted in the identification of a number of further Shuni virus cases in horses as well as in a rhinoceros and a crocodile with neurological disease in South Africa.

MATERIALS AND METHODS

ORIGINAL ISOLATE IDENTIFICATION

CSF of the original case was inoculated on Vero cells and cultured until viral cytopathic affect was visible and was then subjected to electron microscopy. Virus culture stocks were stored at -70°C until RNA extraction. Viral RNA was amplified by a bunyavirus family RT-PCR and PCR products visualised following electrophoresis on a 1.5% gel by UV Transillumination. PCR products were gel purified and cloned using the CloneJET PCR cloning kit (Fermentas, USA). Recombinant clones were sequenced and

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alignments were carried out using the ClustalW. The calculation of genetic distances and construction of Neighbour joining phylogenetic trees based on nucleotide sequence was carried out using MEGA 4 software.

RNA EXTRACTION, AMPLIFICATION AND CLONING

RNA was extracted from blood using the QIA-amp viral RNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's instruction. A 30mg piece of tissue was used for extraction from fresh brain using the RNeasy Plus mini kit (Qiagen, Valencia, CA). Amplification was carried out using newly designed Shuni virus specific primers. RT-PCR products were visualised following electrophoresis on a 1.5% gel by UV Transillumination. PCR products were then gel purified and cloned using the CloneJET PCR cloning kit (Fermentas, USA).

SEQUENCING AND PHYLOGENETIC ANALYSES

Recombinant clones were sequenced and alignments were carried out using the ClustalW. The calculation of genetic distances and construction of maximum likelihood phylogenetic trees based on nucleotide sequence was carried out using PhyML software.

RESULTS

Sequence analysis of the original isolate revealed a 520nt fragment, highly similar to the S segment of the orthobunyaviridae genome. A Phylogenetic tree was constructed, using this 520nt fragment of the S fragment. The Neighbour joining method indicated high bootstrap support (>91%) for the placement of SAE 18/09 within the Shuni, Aino and Kaikular branch. Nucleotide identity was determined using the p-distance model (Nei and Gojobori, 1986) in the distance estimation program of MEGA 4 (Kumar, *et al.,* 2007). Unknown isolate SAE 18/09 was shown to share 95.9% identity to Shuni virus and 91.1% identity to Aino virus. These results indicate that the disease causing agent in the index case as Shuni virus.
During 2010 83 horses and 27 other animals were screened for Shuni virus, of these eight were found to be PCR positive. All positive animals had experienced neurological symptoms and ranged in age from 1-16 years. These cases showed a high sequence identity to Shuni virus, and appear to be more similar to SAE 18/09 than the original Shuni virus isolate described in 1966.

DISCUSSION

In this study we report Shuni virus infection in equines who presented with neurologic symptoms. Phylogenetic analysis indicated that all these Shuni virus cases are very similar at the nucleotide level, with percentage identities ranging between 0 and 3.1%. When compared to the prototype virus, Simbu, percentage identities ranged between 23.5 and 25.9%. The majority of the Bunyaviridae are transmitted by biting arthropods, which develop a lifelong persistent infection. Disease can however also be spread by exposure to infected tissues. Cases of infection with such agents thus have to be monitored to prevent and stop the spread of disease as well as to increase our understanding of the etiology of neurological disease in horses.

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SINDBIS AND MIDDELBURG VIRUSES AS A CAUSE OF DISEASE IN ANIMALS IN SOUTH AFRICA: THE MOLECULAR EPIDEMIOLOGY

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SUMMARY

Alphaviruses are a significant cause of worldwide morbidity and mortality in both humans and animals. In South Africa the most common alphaviruses are Sindbis and Middelburg viruses. In order to determine the contribution of these alphaviruses to unexplained hepatic and neurological disease or fevers in horses and other animals in South Africa, cases resembling these symptoms were screened for Alphaviruses over three consecutive seasons. The results show that both Sindbis and Middelburg virus can contribute to both neurological disease and fevers in horses and rhinoceros.

KEY WORDS:

Sindbis-like virus, Middelburg virus, wildlife, horses, rhinoceros and encephalitis

INTRODUCTION

Alphaviruses are globally widespread and are important pathogens for a variety of animals, insects and fish and are also a major cause of morbidity and mortality in humans[1,2]. In South Africa (SA), Alphavirus infections are mainly due to either Sindbis virus (SINV) or Middelburg virus (MIDV) (*Togaviridae* virus family)[2] and are transmitted by *Culex* and *Aedes*[4] mosquitoes respectively; which are also the primary transmission vectors of West Nile virus (WNV) and Wesselsbron virus[8]. The alphaviruses are divided into two main groups; namely the Old World and New World viruses[11]. Old World viruses result in a febrile, arthritic-type disease in the small joints which may be accompanied by a rash [5, 8, 13] and rarely causes a fatal disease or encephalitis[6]; the New World viruses primarily result in fatal neurological disease[10]. Until the 1974 outbreaks in the Karoo and Northern Cape Province in SA[3] SINV was considered to be of little medical importance[5, 12] due to it being a self-limiting febrile disease. In 1983/84 another epidemic occurred in the Witwatersrand/Pretoria regions of SA, where hundreds of human cases were reported, although mainly febrile[3]. MIDV was thought to be non-pathogenic until it was isolated from the spleen of a horse in Zimbabwe that died of symptoms clinically similar to African Horse Sickness[1]. In SA, serological studies done in animals in the 1960s indicated a high prevalence of both SINV and MIDV on the Highveld, suggesting a relatively high percentage of infection by these viruses in animals**.** As part of a study to investigate mosquito-borne arboviruses as causes of unexplained neurological/hepatic disease or fevers in horses and other animals, acute specimens from horses and wildlife with these symptoms were screened for alphaviruses by molecular techniques.

MATERIALS AND METHODS

SPECIMEN COLLECTION

Blood, cerebrospinal fluid (CSF) and tissue was collected from March 2007 to June 2010, from horses and other animals displaying fever, hepatic and/or neurological symptoms of an unknown aetiology. Samples were received from the Onderstepoort Veterinary Institute (OVI), University of Pretoria Faculty of Veterinary Science and by private veterinarians from around the country.

VIRAL RNA EXTRACTION, ALPHAVIRUS RTPCR AND CLONING AND SEQUENCING

Viral RNA was extracted from blood and tissue using the QIA-amp viral RNA mini and RNeasy Plus mini kits (Qiagen, Valencia, CA) according to the manufacturer's instruction. An alphavirus family-specific nested RT-PCR was used to screen clinical specimens[10] and PCR products were visualised following agarose gel electrophoresis and UV transillumination. Positive alphavirus PCR products were purified, cloned and sequenced according to standard techniques.

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PHYLOGENETIC ANALYSIS

Sequences were aligned and Maximum Likelihood trees generated using PhyML v2.4.4 with a bootstrap consensus tree generated for 100 bootstrap replicates. Nucleotide and amino acid P-distance analysis was carried out with Mega v4.

RESULTS

RT-PCR AND DNA SEQUENCING

A total of 253 equine/livestock/wildlife specimens were screened over 3 consecutive seasons. A majority of the cases submitted were due to unexplained neurological symptoms (66%), while fevers/hepatic disease only accounted for 21% of the samples. Most samples submitted were blood (58%) while tissues only amounted to 39% of the total. Twenty were alphavirus RTPCR positive and 3 were positive by serology (haemagglutination inhibiton or HI). The 20 RTPCR positives consisted of 7 Sindbis-like virus and 13 MIDV samples. Serologically, two serum samples were Sindbis-HI (Sin-HI) positive while one was Middelburg-HI (Mid-HI) positive.

GEOGRAPHIC DISTRIBUTION AND CLINICAL DETAIL

2008

Four alphavirus positives were identified, 2 SINV RTPCR positive and 2 Sin-HI positive. Three horses had single infections with SINV while one RTPCR positive had a co-infection with Equine encephalosis virus (EEV) kaalplaas. Both single and co-infections displayed neurological disease, had fever and were depressed while only 2 horses had congested mucous membranes and swelling of the supraorbital fossae.

2009

Six alphavirus positives were identified, 5 by RTPCR and 1 by serology. Of the RTPCR positives 4 were identified as SINV and 1 as MIDV. One horse was MID-HI positive. Two SINV-like PCR positives and one MID-HI positive had co-infections with WNV while the remaining 3 were single infections. Horses with co-infections were euthanized due to the severity of the neurological disease. The 2 horses with single SINV infections displayed symptoms of colic, jaundice, fever and neurological symptoms but survived; while the horse with the single MIDV infection displayed severe neurological symptoms but recovered.

2010

Thirteen positive alphavirus cases were identified in horses and rhino's; 9 MIDV cases in horses, 3 MIDV in rhino's and 1 SINV in a rhino. Two horses had co-infections, 1 with MIDV and Shuni and 1 with MIDV and EEV. One rhino had a co-infection with MIDV and EEV. Seven horses and two rhino's had a single infection with MIDV while 1 rhino had a single infection with SINV. All horses displayed neurological symptoms, ataxia, incoordination and fever. Two had congested mucous membranes and were slightly leucopaenic. All rhino's displayed neurological symptoms such as weakness and progressive paralysis with death occurring within 24 hours of recumbency.

PHYLOGENETIC ANALYSIS OF ALPHAVIRUS RTPCR POSITIVE CASES

Phylogenetically, the Sindbis-like specimens grouped with the SA strains of Sindbis-like viruses Girdwood and SA.A.R886[13] as well as a Chinese Sindbis-like virus YN87448[7], all of which were associated with a mild febrile disease in humans[5].

Analysis of the NSP4 protein region identified 0.7% nucleotide differences on average within both the horse and rhino Sindbis-like specimens. Both the horse and rhino Sindbis-like strains differed to the known Sindbis-like strains from between 1.4%-2%. However, the rhino Sindbis-like strain differed to the prototype Sindbis virus by 9.6% while the horse strains differed by between 8.2-8.8%.

The positive Middelburg strains differed between 3.4 – 4.8% to the known strain of Middelburg virus in the NSp4 region; and to the known Sindbis-like viruses by 29-32%. On average there was between 0.7 – 4.8% nucleotide differences between strains identified in the present study. The Middelburg strains identified in this study all grouped together and were more closely related to one another than to earlier Middelburg strains[9].

DISCUSSION

Identification of disease causing agents is key to enabling protective measures in terms of handling infected animals and preventing the spread of diseases.
Alphavirus screening over 3 summer seasons identified 20 RTPCR positive samples, 7 of which were Sindbis-like and 13 of which were Middelburg viruses. Severe neurological symptoms and death were only seen in horses that had SINV co-infections with WNV. Identification of SINV in the brains of these horses may be linked to the neurotropic WNV infection, which may have weakened the blood brain barrier (BBB). One horse with a single MIDV infection developed severe neurological disease however; all other horses with single MIDV or SINV infection did not. In comparison, rhino's infected with either SINV or MIDV, whether single or co-infected, displayed severe neurological disease that lead to all animals dying within 24 hours. Horse samples came from all provinces in the SA with ages ranging from 17 months to 14 years. Rhino samples came from Swaziland and the Gauteng and North West provinces of SA with ages ranging between 17 months to 6 years.

CONCLUSION

The Sindbis and Middelburg alphaviruses contribute to unexplained fever and neurological disease in horses and rhinoceros in South Africa and should be considered in the diagnosis of animals with neurological disease or fevers of unknown aetiology. Further epidemiological studies need to be done in order to determine the prevalence of these viruses as well as the need for vaccine development.

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PATHOLOGY OF RECENTLY DIAGNOSED FATAL NEUROTROPIC ARBOVIRAL INFECTIONS IN HORSES IN SOUTH AFRICA

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SUMMARY

This paper is the first account of central nervous system lesions caused by fatal lineage 2 WNV, Sindbis virus and Shuni virus infections in several horses showing severe neurological signs and either death or euthanasia in South Africa. Lineage 2 WNV, prior to 2007-2008, when the first neurological cases in horses were diagnosed, was considered to be nonpathogenic in horses but has since been identified in several fatal cases. Lineage 2 WNV associated lesions of spinal cord and brain appear to be similar to those reported as caused by lineage 1 WNV, with variations in severity, type and CNS localities from case to case and within cases. Two of the horses with neurological signs had co-infections with WNV & the alphavirus, Sindbis. Sindbis virus has not previously been reported as a cause of neurological disease in horses but is neurotropic in mice. The pathology of two cases of Shuniviral equine neurological disease is also presented. Shuni virus, a Simbu serogroup virus of the genus Orthobunyaviridae, was previously diagnosed in one Zimbabwean and one South African horse with severe meningoencephalitis in 1977. The gross pathology and range of histopathological lesions in all cases, despite the viral etiological agent/s involved, were similar , varying only in distribution and severity and with no constant pattern, thus necessitating other diagnostic tests like RT-PCR, viral isolation, electron microscopy and/or immunohistochemistry for specific diagnosis.

INTRODUCTION

The globally widespread flavivirus, West Nile virus (WNV), cycles naturally between birds and ornithophilic mosquitoes, especially *Culex univittatus* in South Africa (Jupp (2001)). Lineage 1WNV has been reported in the northern hemisphere & the closely related Kunjin virus in Australia (Sherret et al (2001), Burt et al (2001)). Lineage 2, had only been reported in Southern Africa and Madagascar (Burt et al (2002), Jupp (2001)) until 2004 & 2005, when it was discovered in raptors (Bakonyi et al (2006)) and in 2008 in horses (Kutasi et al (2009)) in Southern Hungary. Southern African WNV strains were previously thought to cause only mild occasional flu-like disease in humans (McIntosh et al (1976), Jupp et al (1986), Jupp (2001)), and to be non-pathogenic in birds (McIntosh et al (1976), Jupp (2001)) and horses (Guthrie et al (2003)). There were, however, records of single fatalities in a dog, an ostrich chick, a Thoroughbred foal, and a human north of Pretoria (Burt et al (2002)).

Subsequent WNV studies in mice showed some lineage 2 strains to be as neuroinvasive as lineage 1 strains (Venter et al (2005), Beasley et al (2002)). A survey of horses with neurological signs begun in summer of 2007-2008 (Venter et al (2009)) showed 7 horses to be WNV lineage 2 positive by RT-PCR on blood, brain and/or spinal cord: 5 died or had to be euthenased & brief pathology descriptions were given of 3.

Sindbis virus is a group A arbovirus antigenically closely related to Western equine encephalitis (WEE) virus (Johnson (1965)), an alphavirus present in western North & Central America & several South American countries which causes neurological disease in especially horses and humans. Sindbis virus had been recovered from *Culex* spp mosquitoes in Africa, Asia, Australia (Johnson (1965)) and Europe (Francy et al (1989), Gibbs (2004)). It shares the same ecology as WNV in Southern Africa (Jupp (2001)), and causes similar flu-like disease, myalgia, arthralgia and skin rash in humans as does WN fever. As of 1965, no human cases of Sindbis related encephalitis had been reported (Johnson (1965)) and no reports of naturally-occurring neurological disease in horses or other animal species were found in searches of current literature. In suckling mice Sindbis virus induces fatal encephalitis after either intracerebral or extracerebral inoculation (Johnson (1965)).

Shunivirus, an arbovirus of the Simbu serogroup of the family Bunyaviridae, genus Orthobunyabirus, was originally found in cattle or goat sera and later in midges in Nigerian surveys conducted from 1964 to 1969 (Causey et al (1972)). In 1977, Shunivirus was isolated from brains of two horses, one from Zimbabwe (St George et al (2004)) and one from South Africa (Coetzer & Howell (1998)), submitted for Rabies virus examination, which was negative: both had severe meningoencephalitis. Shuni virus has been isolated from *Culicoides* midges in Nigeria and mosquitoes in South Africa (St George et al (2004)).

This paper reports the histopathological presentation and post-mortem findings of a selection of fatal cases caused by these viruses and identified over the past 3 years in horses in South Africa

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MATERIAL AND METHODS

Eight horses, sampled from 2008 to 2010 summer seasons, with severe neurological signs, which either died or were euthenased, and which were diagnosed with either single WNV infection (n=4), WNV co-infection with Sindbis virus (n=2), or with Shuni virus infection (n=2), were necropsied and CNS specimens preserved in 10% formalin for histopathological examination. RT-PCR, viral isolation and electron microscopy were variously performed on fresh CNS specimens or infected tissue culture cells from these cases.

RESULTS

All cases were positively diagnosed by specific RT-PCR of fresh CNS specimens taken at the time of the necropsies. One WNV and one Shuni case also had successful viral isolation obtained from fresh CNS tissue with subsequent RT-PCR. Bunyavirus particles were visualised & identified ultrastructurally in infected tissue culture cells prior to PCR identification of one Shuni positive case. Two WNV cases had rare flavi-virus positive immunohistochemical staining of neural processes in distal spinal cord. All WNV positives were sequenced as lineage 2.

The gross pathology common to most cases, despite etiology, was lung oedema and congestion, with variable presence of hydropericardium or other cavity fluids, subcutaneous and other regional oedema, and mild surface or cardiac haemorrhages. These findings, together with oedematous supraorbital fossae present in some cases, mimicked gross lesions of African Horsesickness and Equine Encephalosis, *Culicoides* sp transmitted orbiviral diseases present in South Africa. Macroscopically visible multifocal haemorrhages and/or markedly distended vessels occurred in spinal cord and/or especially lower brain centres of the 2010 WNV cases. Histopathological lesions most commonly found in spinal cord sections were assymetrical, not always bilateral and included haemorrhages; vascular congestion especially of grey matter but also multifocal distended white matter vessels; perivascular cuffing with mononuclear cells and occasional neutrophils - most prominent in ventral horn grey matter but sometimes occurring in dorsal and lateral horns and elsewhere; variable mononuclear meningitis and occasional vascular wall necrosis; gliosis; glial nodules which at times contained neutrophils; occasional poliomalacia; & occasional neuronal chromatolysis or necrosis.

Lesions similar to those found in spinal cord occurred in the brain and cerebellum, but especially affecting lower white matter regions. Olfactory lobe lesions in one of the single WNV cases were outspoken. One of the WNV-Sindbis co-infection cases which had been down for 2 weeks prior to admission, had minimal inflammation in cerebral white matter, and the presence of perivascular collagen suggested healing of prior inflammation. Meningitis was a variable lesion, with the most severe findings affecting the cerebellum in one Shuni & one WNV case. Some animals had more prominent distal spinal cord lesions, and others had worse cranial cord lesions.

The 3 WNV single infection cases from the late 2010 summer season, despite showing similar severe neurological clinical signs to previous cases, had macroscopically visible and comparatively severe lesions microscopically in the CNS; the lesions from the 2008 WNV case (Venter et al (2009)) and the 2009 WNV/Sindbis co-infections were comparatively of only mild to moderate severity. One of the Shuni positive cases had severe multifocal inflammation affecting brain, cerebellum and proximal spinal cord, whereas the other Shuni positive case showed congestion, haemorrhages and oedema of cerebrum and cerebellum without inflammatory cells, possibly reflecting different stages of infection. Cerebellar Purkinje cell degeneration and loss were noticeable in the Shuni cases.

DISCUSSION

The array of lesions found in these cases was similar to those listed in northern hemisphere lineage 1 WNV equine nonsuppurative polioencephalomyelitis (Schmidt et al, (1963), Cantile et al (2000 & 2001), Guillon et al (1968), Bunning et al (2002 & 2004), Snook et al (2001), Autorino et al (2002), Steinman et al (2002), & Tber Abdelhaq (1996)). The South African cases showed much variation of CNS pathology between and within individual cases.

The WNV co-infections with Sindbis, and single Shunivirus infections appeared histologically similar in many respects to single WNV infections, making diagnosis on histopathology alone impossible. Without good immunohistochemical techniques it would be impossible to ascertain the extent & pathology caused by either Sindbis or WNV virus in CNS tissues of the co-infection cases, or to know whether one exacerbated the other.

Shunivirus has not been reported as a cause of neurological disease in humans to date, however, arboviruses have not routinely been investigated in humans with neurological disease in South Africa. Since the 1977 equine cases had severe neurological signs and encephalitis, and the current equine cases were single Shuniviral infections on RT-PCR testing, ultrastructurally or culture of

fresh CNS, it can be confirmed that Shuni virus can cause severe fatal neurological disease in horses, and should be further investigated in man, horses & other species in South Africa.

The environmental factors, especially seasonal high rainfall and warm temperatures, promote insect proliferation including mosquitoes and midges which transmit the viruses reported in this paper, & single or co-infections of incidental mammalian hosts with these arboviruses are therefore explained. It remains crucial that animals and man with neurological disease be diagnostically screened for these and possibly other viruses with the view to development of effective preventative and therapeutic measures.

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IMPROVED DETECTION OF AFRICAN HORSE SICKNESS VIRUS IN NATURALLY INFECTED HORSES: A PROSPECTIVE STUDY

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SUMMARY

African horse sickness (AHS) is a life threatening disease of equids and has a significant impact on the equine industries of South Africa. It is caused by African horse sickness virus (AHSV), a member of the genus *Orbivirus* in the family *Reoviridae*. It is transmitted to horses by *Culicoides spp.* and is therefore most prevalent during the time of year, and in areas where the *Culicoides spp.* are most abundant, namely in late summer in the summer rainfall areas of the country. Whilst the clinical signs and presentation of the disease were well documented by Sir Arnold Theiler, very little is known or documented about the clinico-pathology of the disease.

In this study, which started in February 2009, serum, heparinized and EDTA blood samples were collected and rectal temperatures recorded weekly from 50 Nooitgedacht ponies resident in open camps at the Faculty of Veterinary Science, Onderstepoort. The horses ranged in age from foals a few days old at the start of the project, to horses 26 years of age. The blood samples were tested for the presence of AHSV dsRNA by a recently developed real-time reverse transcription polymerase chain reaction assay (RT-PCR) (Quan, Lourens, MacLachlan, Gardner & Guthrie (2010)). Positive samples were evaluated further by measuring haematological parameters (specifically thrombocyte numbers). The clinical picture of the AHSV-positive horse was recorded, rectal temperatures recorded daily and clinical cases treated at the Onderstepoort Veterinary Academic Hospital (OVAH) with a standard protocol.

INTRODUCTION

African horse sickness is an important economic disease in southern Africa due to the restrictions on international equine trade from southern Africa to the rest of the world. Horses are the most susceptible equids to AHSV infection, with mortalities of up to 90% in fully susceptible animals (Guthrie & Quan (2009)). Foals born to immune mares acquire passive immunity by ingestion of colostrum after birth. Antibodies against AHSV decline progressively to undetectable levels by four to six months of age (Coetzer & Guthrie (2004)). The clinical signs and presentation of infection with AHSV were documented comprehensively by Sir Arnold Theiler (Theiler (1921)). The disease is characterised by pyrexia, oedema of the lungs, pleura and subcutaneous tissues, as well as petechiae and haemorrhages (Kazeem, Rufai, Ogunsan, Lombin, Enurah & Owolodun (2008)). The incubation period was found to be, on average, five to seven days in experimental cases (Coetzer et al. (2004)).

A number of polymerase chain reaction (PCR) assays have been developed for the detection of AHSV. Advantages to this approach are that they have the potential to be rapid, sensitive and versatile, and can supplement the older conventional methods. It can be used on specimens that do not contain live virus (Coetzer et al. (2004), Agüero, Gómez-Tejedor, Cubillo, Rubio, Romero & Jiménez-Clavero (2008)). A real-time polymerase chain reaction assay (RT-PCR) has been developed recently (Quan et al. (2010)). This assay has been characterised using field strains of African horse sickness, which has never been done with an AHS PCR before.

In 2006, an outbreak of AHS occurred in the AHS protection zone in Robertson, Western Cape (ProMED-mail (2006)). It was suspected that a subclinical viraemic horse that had been transported from Gauteng into this area was the source of this outbreak. This outbreak made it clear that field studies in AHS needed to be conducted in order to get a better understanding of the dynamics of the virus in the field. Up to this point only experimental studies involving the AHS viraemia have been done. Furthermore, all the PCR studies have been experimental, and not in the field situation.

The aim of our study was to follow a herd of horses in an AHS endemic area longitudinally during the AHS season. We aimed to study the dynamics of the virus in naturally infected horses under field conditions. We established that subclinical cases of AHS occur naturally under field conditions. We also documented the dynamics of thrombocyte counts in AHS cases. Furthermore, the dynamics of RT-qPCR results were described in these cases.

MATERIALS AND METHODS

Fifty horses from the Onderstepoort Teaching Animal Unit (OTAU) herd, with known AHS vaccination history, were used in this study. The rectal temperature was recorded and EDTA and serum blood samples collected by jugular venipuncture from each horse on

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a weekly basis during the 2008/2009 and 2009/2010 summer seasons. The EDTA sample was tested for the presence of AHSV dsRNA with a real-time RT-PCR (Quan et al (2010)) and the serum sample stored at -20°C.
Animals that tested positive for AHSV had EDTA and heparinised blood samples collected and the temperature, pulse and respiratory rates of each horse was recorded. Viral isolation on BHK-21 tissue culture (Quan, Van Vuuren, Howell, Groenewald, & Guthrie (2008)) was attempted from the heparinised blood sample and a complete blood count was done by the Onderstepoort Clinical Pathology Laboratory using a veterinary blood cell counting analyser (Cell Dyn 700) and AHSV dsRNA levels determined (Quan et al (2010)) from the EDTA sample. The following sampling schedule was followed: blood samples were collected and data recorded from non-pyrexic animals (i.e. temperatures not exceeding 39°C at any sampling time) daily for five consecutive days, after which sampling was reduced to every Monday and Thursday until three consecutive samples were negative for AHSV, as determined by real-time RT-PCR. From pyrexic animals (i.e. temperatures exceeding 39°C at any sampling time), blood samples were collected and data recorded daily until the temperature of the horse dropped below 39°C, after which the sampling protocol for non-pyrexic animals was followed.
Vaccination against AHSV and the treatment protocol for AHS was done according to the Standard Operating Procedure of the OTAU. All horses included in this study, excluding new born foals, had been vaccinated at least once with the Onderstepoort Biological Products polyvalent vaccine prior to the AHS season.

RESULTS

Of the 50 horses sampled during 2009 and 2010, seven tested positive for AHSV during the 2009 summer, and two during the 2010 summer. Of the nine laboratory positive cases, two were moderate clinical cases of "dikkop". One of these horses, Kotie, a three year old mare, presented during the weekly sample collection with a fever of 40°C, a thrombocyte count of 44.5×10^9/L, the normal minimum count being 117×10^9/L (Blood & Studdert (1999)), and a real-time RT-PCR cycle threshold (C_T) value of 25.72. Over the following three days, moderate periorbital swelling developed, together with a mild increase in respiratory effort and a minimum thrombocyte count of 9.3×10^9/L was obtained. These symptoms subsided gradually over two weeks, together with a decrease in viraemia and the thromobocyte count normalised six days after detection of AHSV dsRNA in the blood. AHSV dsRNA was present in the blood for more than 120 days after the spike in temperature.
Jenny, a two year old mare, was a moderate clinical AHS case. She tested positive by real-time RT-PCR with a minimum C_T value of 27 over the next week and remained positive for a 140 days. She presented with a platelet count of 42×10^9/L which increased over the following four days until it was in the normal range. She developed similar symptoms to Kotie over a comparable time period.
One foal suffered from the "dunkop" form of African horse sickness. A C_T value of 21.76 was obtained during the weekly sample testing even though the foal showed no clinical abnormalities. The following day it was found collapsed in the camp showing severe dyspnoea, cyanotic mucous membranes and was seizuring. It was euthanased later that day due to lack of response to treatment. This foal had a platelet count of 122×109/L on the day it died.
There were 2 mild clinical cases, most likely the horse sickness fever from the disease, with the following clinical signs: a rise in average body temperature, mild supraorbital swelling and a drop in platelet count. Samples from Cazza, a 4 year old mare, showed a minimum platelet count of 57×10^9/L and a minimum C_T value of 26. She was real-time RT-PCR positive for 28 days. Samples from Demi, a four year old mare, showed a minimum platelet count of 64×10^9/L, and a minimum C_T of 27. She remained real-time RT-PCR positive for greater than 60 days. The habitus of these 2 horses remained normal throughout this period, and these cases would more than likely be missed in the field.

There were 4 subclinical cases. These horses showed no rise in their average temperature and their platelet counts did not drop below the normal limit. They showed no change in their habitus, and no other clinical abnormalities. They remained real-time RT-qPCR positive for between 30 to 40 days.

DISCUSSION

A novel and important finding from this study is the detection of subclinical cases of AHS (detection of AHSV by real-time RT-PCR in the absence of clinical signs). This RT-PCR assay has been applied to samples collected from clinical cases of AHS and has been applied to serial samples collected from animals experimentally infected with AHSV. In one case, a single horse that had not been vaccinated against AHSV previously and was challenged with a virulent AHSV strain, tested positive using the RT-PCR assay seven days after challenge and tested positive for in excess of 100 days post challenge. This animal had pyrexia in excess of 39°C from day 12 to day 14 post challenge. The viraemia was thus detectable by RT-qPCR approximately 5 days prior to the animal becoming pyrexic. In the same study, one of 14 vaccinated horses that were challenged with the same virulent AHSV strain tested positive from day seven and remained positive until day 37 post challenge (Guthrie, personal communication). This preliminary data from this

single vaccinated horse provides support for the hypothesis that horses that have been vaccinated against AHSV can be subclinically infected with AHSV if challenged.

A total of nine cases of AHS were identified in the 50 horses included in this study (18%). Of these, three (6%) presented as clinical AHS and six (12%) were mild clinical or subclinical cases. The minimum C_T values of these mild clinical to subclinical cases ranged from 26 to 34, and similar values are often obtained from blood samples collected from clinical AHS cases. At present the viraemia required for horses to infect midges is not known. The appreciable viraemia detected in the mild clinical and subclinical cases suggest that these animals may have a viraemia sufficient to infect midges. This being the case, such cases pose a severe risk for introduction of AHS into an area if they are moved during this period of subclinical viraemia.

As has been reported in experimental cases of AHS, the RT-PCR detected AHS in horses prior to the onset of clinical signs. This was particularly noticeable in the foal that was clinically normal with a CT of 21 the day prior to its euthanasia. The most likely explanation for the fact that the platelet count was not below normal in this foal is that it died before a drop in the count was realised.

Horses with AHS often present with a decreased thrombocyte count. In this study, where viral load was quantified using RT-PCR, the drop in thrombocyte count appeared to follow on the drop in C_T.

In all cases that survived, AHS derived nucleic acid could be detected for extended periods. In the clinical cases AHS was detected for more than 60 days. Mild clinical to subclinical cases tested positive for 30 to 40 days. AHS can be isolated from the blood of an infected horse for up to 21 days (Coetzer et al (2004)). AHS nucleic acid was detected in the blood for periods considerably longer than this. The period which horses can be infectious to midges is not known but may extend beyond the current estimated viraemic period. This warrants further investigation.

In this study we were able to establish a pattern in the thrombocyte counts of infected horses. This drop in thrombocyte count along with factors such as the minimum C_T value may be useful as prognostic indicators and should be further investigated. We have seen that horses may remain viraemic and therefore infectious to midges for far greater periods than originally estimated. Most importantly, we have demonstrated that naturally infected, subclinical cases of AHS do occur and this critical finding may influence the way we manage the risk of AHS associated with movement of horses. These risks require further investigation.

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DEVELOPMENT OF AN AHSV VP7/WNV ENVELOPE DOMAIN III RECOMBINANT AS A SUBUNIT VACCINE CANDIDATE FOR PROTECTION AGAINST WEST NILE VIRUS.

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INTRODUCTION

West Nile virus is a member of the flavivirus genus in the flaviviridae family (Bonafe *et al.*, 2009). Two major lineages are recognised, lineage 1, circulating in North America and Australia and lineage 2 circulating in Southern African regions (Brault *et al.*, 2004). Recent investigations have highlighted lineage 2 WNV as an important cause of neurological disease in horses in South Africa that may be fatal in > 60% of cases (Venter *et al.*, 2009).

A great deal of progress has been made towards the development of West Nile virus vaccines. An inactivated vaccine is available for horses against lineage 1 WNV strains and has been in use since 2002 in the USA. This vaccine has largely decreased the amount of equine infections in North America (Granwehr *et al.*, 2004). Other veterinary related vaccines are at various stages of development ranging from basic preclinical tests to more complex clinical animal trials (Monath *et al.*, 2006). These vaccines consist of lineage 1 antigens and have been shown to induce a protective immune response against lineage 1 strains. Although the above mentioned vaccines are currently licensed for use in horses in the USA and Europe, these vaccines are not yet licensed in South Africa. Although the possibility of providing a degree of cross protection between different flaviviruses has been investigated, cross protection studies for the different WNV lineages have not yet been carried out (Monath *et al.*, 2001). A recent recall of recombinant live WNV-yellow fever vaccines in horses in the USA has raised concerns about the safety of live attenuated vaccines of this nature in horses and may delay licensing of such vaccines in South Africa. Advances in recombinant vaccine technology have overcome the limitations of conventionally prepared vaccines. Investigations into the development of recombinant subunit vaccines have increased as this approach has proven to have several advantages over live or inactivated vaccine (Liljeqvist, Stahl, 1999). To enhance the immune response that is elicited from such recombinant vaccines; improved adjuvants and better delivery vehicles are investigated. A more efficient immune response is stimulated when a soluble, bioactive protein is recovered during the production of an antigen (Singh, Panda, 2005).

A majority of the above mentioned experimental studies have focused on incorporating the envelope glycoprotein of WNV as part of a novel sub unit vaccine design. This protein is the primary immunogen and has proven itself as an efficient vaccine candidate for the protection against lethal WNV infections (Volk *et al.*, 2004). The WNV E protein contains numerous hydrophobic regions some of which is found on the C-terminal region (Kanai *et al.*, 2006). In order to produce a more soluble glycoprotein, the C-terminal portion is removed when expressing the WNV E protein (Ledizet *et al.*, 2005). .

The WNV E structural glycoprotein is composed of three antigenic domains. Several neutralising epitopes have been mapped to the surface of domain III (Oliphant *et al.*, 2006). Highly potent immune responses have been induced when administering this antigen in mice (Chu *et al.*, 2005).

African horse sickness is a major horse pathogen in South Africa (Alexander *et al.*, 1995). Currently protection is provided by a live attenuated vaccine that provides protection against most of the 9 serotypes although the nature of the vaccine is not ideal for competition horses and adverse events have been reported. Vaccination with the inner core protein VP7 has been shown to provide partial protection against challenge with AHSV, that is likely group specific. Serotype specific protection is afforded by the outer core protein VP2 (Stone-Marschat *et al.*, 1996). It is suggested that protection provided by VP7 may be cellular of nature (Wade-Evans *et al.*, 1997). Currently AHSV remains a major problem in the country and outbreaks of strains not currently included in the vaccine are reported annually. An updated vaccine that protects against all AHSV strains is urgently needed, but a safe booster vaccine that enhances protection afforded by the current vaccine will also be beneficial.

A novel antigen presentation system has been developed that makes use of the VP7 protein from the AHSV virus particle. This protein has been expressed in the baculovirus expression system and has been altered to allow various immmunogens to be incorporated into its top hydrophilic loop region. This platform has improved on the method of vaccine delivery, antigen presentation and protein solubility, which may enhance the induction of an efficient immune response.

AIM

The long term aim of this study is to produce a chimeric vaccine that makes use of the major core particle protein of African Horse sickness virus (VP7) as a novel antigen presentation system for the West Nile virus E-protein neutralisation domain III. Here the development of the vaccine construct and improvements to the antigenic properties through increasing the solubility of the recombinant protein.

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METHODS AND MATERIALS

The Baculovirus expression system was used to produce chimeric AHSV VP7 mt144–WNV EIII proteins with lineage 2 E-proteins. Three additional controls were expressed including the wild type AHSV VP7 protein, domain III of the WNV envelope protein (separate from the VP7 vector) and the ectodomain of the WNV envelope protein
Recombinant baculovirus particles were generated and each recombinant protein was expressed in insect cells. The expression of each protein was confirmed through SDS-PAGE analysis. Western blot analysis with mouse WNV polyclonal antibodies was used to confirm authentic expression of recombinant WNV envelope proteins (including the chimeric AHSV VP7 mt144-WNV EIII).
To investigate and compare each construct according to its solubility, proteins were analysed on a 50% - 70% sucrose gradients and ultracentrifugation. Post harvest treatment strategies with arginine and sarkosyl, were used to improve the solubility of recombinant proteins.

RESULTS

Target genes were successfully cloned and expressed with the use of the baculovirus expression system. High levels of protein expression were confirmed by PAGE analysis against negative controls. All constructs containing WNV proteins were recognised by mouse polyclonal antibodies following western blot analysis. Anti-WNV mouse antibodies confirmed the presence of each antigen, including the chimeric protein AHSV VP7 mt144-WNV E_{III}, in all vaccine constructs but was not recognised in the AHSV-VP7 protein alone.
Sucrose gradient analysis showed that protein products were insoluble, however several post harvest treatment strategies have been used to overcome this. Treatment with both Arginine and sarkosyl produced a soluble component for each recombinant protein. These treatment strategies are now being used to purify soluble proteins.

CONCLUSION

Soluble protein has been shown to be necessary for the induction of an efficient immune response (Huismans, 2006). Administering such antigens promotes an effective neutralising antibody response. A high level of protein expression was achieved. Such levels of protein production often results in the accumulation of insoluble proteins which cannot be used as vaccines (Singh, Panda, 2005). Post harvest treatment of each recombinant protein allowed us to generate soluble antigens which may be suitable for further investigation as sub unit vaccine candidates. WNV E_T and WNV E_{III} have both proved to result in a very good immune response by themselves (Chu *et al.*, 2007; Oliphant *et al.*, 2007). Expression on the AHSV-VP7 vaccine vector may enhance this response in animals, but could also have the added advantage of boosting a cellular response against AHSV in horses. These immunogens will now be used in mouse immunisation studies to investigate their neutralisation and protective properties following challenge with lineage 2 WNV.

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CAN BOVINE TUBERCULOSIS HIDE IN THE BEEF AND GAME MEAT WE EAT?

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SUMMARY

The risk for humans to contract bovine tuberculosis through the consumption of undercooked meat (beef or game) as well as biltong (traditionally dried game meat) is a concern. The survival of Mycobacterium bovis (M. bovis) during the cooking and drying processes was investigated in two studies to determine the longevity of M. bovis in 1. spiked bovine organ and muscle tissue (8x107cfu/ml-1) and 2 naturally infected organ and muscle tissues of African buffalo and greater kudu. All tissues were exposed to cooking (10 and 20 minutes respectively) and the muscular tissue to the drying process (biltong), prior to culture. All Mycobacterium spp. isolates were analysed by polymerase chain reaction for the presence of M. bovis.
The results showed that spiked bovine organ samples subjected to cooking for either 10 or 20 minutes were found to yield viable M. bovis in muscle, lymph tissue, liver, kidney and heart tissues, whereas the drying process yielded none. All naturally infected tissues from game subjected to cooking were found negative for *M.bovis* but non-tuberculous mycobacteria (NTM) were isolated from kidney, liver, heart and lymph nodes tissues. The drying process yielded no mycobacterial growth.

KEYWORDS

Mycobacterium bovis, buffalo, kudu, cooking, drying, zoonotic risk non-tuberculous mycobacteria, NTM.

INTRODUCTION

M. bovis is the causative agent of bovine tuberculosis in an extremely wide host spectrum including a range of domestic animals, wildlife and humans and the infection has been stated as a cause of public health concern in Africa[2]. Despite the paucity of information on *M. bovis* in Africa, there is sufficient evidence to suggest that it is widely distributed and is found at significantly high prevalence in some domestic and wild animal populations[5].
In South Africa, the public's exposure to tuberculosis from animals is addressed by the National Bovine Tuberculosis control scheme[4] and by the national meat inspection system in all registered abattoirs. However, animals with non-visible or small and localised lesions could pass through the inspection system unnoticed, or enter the food chain when meat is obtained from uncontrolled sources (not slaughtered in an abattoir). Currently game meat available on the local market (especially in the hunting season – winter) is not subjected to any inspection or other legislative control procedures and this unknown health status motivated the studies presented. The aim therefore, was to determine whether *M. bovis* can survive the secondary processes of cooking and drying (as in the case of biltong) in spiked bovine and naturally infected game organs and muscle tissues.

MATERIALS AND METHODS

ANIMALS

Tissues from buffalo were collected as part of the bovine tuberculosis management program of the Hluhluwe iMmfolozi Park in the KwaZulu-Natal province of South Africa, whereby between forty and eighty buffalo per selected buffalo herd were captured in a mobile boma and tested for bovine tuberculosis using the intradermal tuberculin test. Test negative buffalo were released as a herd and buffalo that tested positive for M. bovis were culled and examined for tuberculous lesions in the abattoir in Hluhluwe iMmfolozi Park by qualified meat inspectors under supervision of the Park`s veterinarian. Greater kudu (in the Hluhluwe iMmfolozi Park) with visible, characteristic abscessation of the parotid lymph nodes consistent with the findings reported by Keet et al. (2001) were assumed to be infected with M. bovis and were culled at night with head shots during a routine disease surveillance programme and followed up by meat inspection as was done for the buffalo at the abattoir.
Eight beef carcasses were sampled on the slaughter floor directly after the primary meat inspection point.

SAMPLE COLLECTION AND PROCESSING

A sample size that allowed for a minimum of 20g of each of the following tissue types: muscle (diaphragm), kidney, liver, heart, lung and lymph nodes (*Lnn mandibulares* and *parotideus*) were collected from each of the carcasses of 8 cattle, 7 buffalo and 7 greater kudu. For every carcass an additional sample was taken from the muscle tissue to allow for the drying process experiment. At the ARC-OVI Tuberculosis Laboratory, samples were frozen to -18 °C (without compromise to *M. bovis*) within 4 hours and stored until analysed. The bovine tissue samples were spiked with $8x10^7 cfu/ml^{-1}$ of a field isolate of *M. bovis* by injecting 1ml of the *M. bovis* suspension into multiple sites in each sample using sterile syringes and needles.

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ESTABLISHMENT OF COOKING TIMES AND A BILTONG RECIPE

The prepared samples were cooked at 100°C in 150 ml sterile distilled water for 10 minutes in earthenware containers and then divided into two equal-sized portions using sterilised scissors. One of these samples was cooked for a further 10 minutes. The biltong making entailed the cutting of meat strips from the diaphragm (20 g of each animal), the curing (12–18 hours) thereof in a standard game biltong mixture of salt, sugar, vinegar and spices and subsequent drying in a class II biohazard cabinet (reduction of a_w) (C. Steynberg 12 June 2004). All of this led to a hurdle effect of preservation (salt, sugar, pH and drying) as more than one preservation barrier is used to inhibit micro-organisms[6,7].

CULTURE AND IDENTIFICATION OF MYCOBACTERIUM SPECIES

All methods for culture and identification of mycobacterial colonies were used as described[8].

RESULTS

BACTERIAL ISOLATION

BILTONG-MAKING

No *Mycobacterium* was isolated from any of the muscle tissue samples from cattle, buffalo or kudu exposed to the biltong-making process (drying) (Tables 1 and 2). However, colony growth was observed on the untreated control muscle tissue samples (not subjected to the biltong making process) and was confirmed by means of the PCR test as *M. bovis*.

Table 1: Isolation of non-tuberculous mycobacteria from buffalo and kudu tissues cooked for different periods of time

Tissue Types	Cooking time	Buffalo	Kudu
		Nr. culture + tissue samples / Nr. (slants)	Nr. culture + tissue samples and / Nr (slants)
Lung	10	0/(0)	0/(0)
	20	0/(0)	0/(0)
Muscle	10	0/(0)	0/(0)
	20	0/(0)	0/(0)
Lymph node	10	5/(5)	0/(0)
	20	4/(3)	0/(0)
Liver	10	2/(7)	0/(0)
	20	2/(2)	0/(0)
Kidney	10	1/(3)	1/(1)
	20	0/(0)	0/(0)
Heart	10	2/(2)	0/(0)
	20	2/(4)	0/(0)

COOKING

Naturally infected tissue samples from buffalo and kudu

No *M. bovis* was isolated from any of the cooked tissues from naturally infected buffalo and kudu. However, non-tuberculous mycobacteria (NTM) were isolated from 1 kidney sample, 2 liver samples, 5 lymph node samples and 2 heart tissue samples from buffalo and 1 kidney sample from kudu (Table 1). The pooled control sample (mixture of buffalo and kudu tissue not exposed to the cooking process) yielded growth which was confirmed as *M. bovis* by means of the PCR test. *M. bovis* was isolated from all spiked bovine tissues except for lung.

Table 2: Isolation of *M. bovis* from different spiked bovine tissue types

Tissue Type	No. carcasses	Cooking time	No. *M. bovis* positive slants/total no. slants
Lung	8	10 min	0/48
		20 min	0/48
Muscle	8	10 min	2/48
		20 min	3/48
Lymph node	8	10 min	1/48
		20 min	1/48
Liver	8	10 min	2/48
		20 min	1/48
Kidney	8	10 min	1/48
		20 min	0/48
Heart	8	10 min	3/48
		20 min	3/48
Total	48		17/576

DISCUSSION

The results showed that *M. bovis* could not survive the drying process of muscle nor the cooking process in spiked lung tissue but was well isolated from especially heart and muscle tissue and to a lesser extend from liver and lymph nodes. A possible explanation for the findings is the higher density of muscle tissues as compared to soft tissues like lung. In naturally infected tissues from buffalo and greater kudu neither the drying nor the cooking processes applied, permitted the survival of any detectable levels of *M. bovis* while it was possible to culture *M. bovis* from the untreated organ samples. However, non-tuberculous mycobacteria (NTM) were isolated from kidney, liver, heart and lymph nodes of 4 out of 7 buffalo and one out of 7 kudu. While NTM occur predominantly in the environment and are mostly non-pathogenic, some species can cause or contribute to disease in individuals with immunosuppression[1]. Although it is not possible to determine the significance of these findings in terms of a veterinary public health risk as the NTM were not speciated, they are certainly a reason for concern. The study has shown that NTM can not only occur in high numbers in game meat but they can survive in food after cooking.

In summary, the consumption of biltong and cooked meat from animals infected with *M. bovis* is considered to pose a minimal risk for infection of humans with this organism, but the recovery of NTM surviving the cooking process warrants further investigation.

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PARTICIPATORY RISK ANALYSIS TO ENSURE FOOD SAFETY OF EDIBLE OFFAL FROM GAME MEAT

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SUMMARY

The meat obtained from trophy hunting, culling and harvesting indigenous game animals has been used in the past by biltong hunters, local butchers and for export markets. The edible by-products, currently discarded, could be used as a renewable source of protein in low income communities. Constraints to the utilization of such edible by-products of game meat include lack of recognized food value chains in informal markets, food safety concerns and market access to edible by-products from game harvesting operations. Participatory risk analysis was used to investigate the feasibility of using edible offal as a source of renewable protein, to address food security issues in poor communities bordering game parks in South Africa. The market outlet for the food value chain selected for investigation was informal markets in Pongola, KwaZulu Natal. In order to promote food safety, only edible offal from impala carcasses that had been through primary meat inspection, were considered during the study. Samples were submitted for microbiology, in line with published EU guidelines and Veterinary Procedural Notices to an accredited laboratory. Structured and informal interviews were held with stakeholders, including veterinarians, game harvesters and informal traders. Scenario planning and decision tree analysis was used to develop a practical food marketing chain and estimate critical control points for identified physical, biological and chemical hazards. In addition, environmental risks were considered and a risk mitigation strategy designed, to reduce any possible environmental impacts. It was concluded that the food marketing chain is feasible and that the current practice of leaving the offal for predators was likely, if it continues, to cause imbalances in the predator/ prey ratio on game farms.

INTRODUCTION

Food scarcity is a contemporary challenge, in rural communities in South Africa, with many child headed households and the need for a protein rich diet for immuno-compromised individuals afflicted by either nutritional diseases or HIV/AIDS. In many parts of the world, offal items are a very popular kind of food that is of central economic interest for meat producers (Riley et al., 1989). So called "red offal" comprises the lungs, liver, spleen and kidneys, whilst other offal includes stomach and intestines as well as sometimes other discarded organs (Gill & Jones, 1992). The visual quality and final microbiological load of offal is determined by the processing method applied. This also determines the eventual shelf life, the consumer acceptability and the consumer risk connected to the final product (Bensink et al., 2002).

"Street foods" can be defined as "foods and beverages prepared and/or sold by vendors (informal traders) in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation" (WHO, 2009)[1].The sale of food items on the street is commonly practiced in many countries of the developing world (Dawson & Canet, 1991) (Umoh & Odoba, 1999). In South Africa, ready-to-eat foods are sold by informal vendors at locations such as railway or bus stations and "taxi ranks" (Mosupye & Von Holy, 2000). Currently, street food vending can be assumed to be the single largest employer in the informal sector of South Africa and is a major contributor to the economy of the country (Von Holy & Makhoane, 2006). However, street food vendors may not meet all food safety and hygiene requirements. Shelters, running water, washing facilities as well as toilet facilities may be inadequate (FAO & WHO, 2005b). (CARDINALE et al., 2005, Ekanem, 1998). Nevertheless, some studies revealed that food prepared on the streets can also be safe and, in such cases, provide sustainable alternative outlets for consumers (FAO & WHO, 2005b; Martins & Anelich, 2000; Von Holy & Makhoane, 2006).
Food safety can be defined as "all conditions and measures that are necessary during the production, processing, storage, distribution and preparation of food to ensure that, when ingested, it does not represent an appreciable risk to health" (Henson, 2003; Miyagishima *et al.*, 1995). A food related risk is defined as "a function of the probability of an adverse effect and the magnitude of that effect, consequential to a hazard in food" (Rooney & Wall; 2003 Schlundt, 2002). In the context of food safety, risk assessment is a structured and science-based process to determine the risk associated with any type of biological, chemical or physical hazard in food. The overall objective is the provision of estimates on the probability of disease occurrence through the characterization of the nature and likelihood of harm. Typically, this comprises qualitative as well as quantitative information and brings along a certain degree of scientific uncertainty (WHO, 2009). The risk assessment process has four major steps, namely hazard identification, hazard characterization, exposure assessment and risk characterization (Schlundt, 1999; WHO, 2009). The JEMRA (Joint Expert Meeting on Microbiological Risk

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2. WHO website Http://www.who.int accessed online December 2009

Assessment) was established by the FAO and WHO to facilitate the provision of expert advice on microbiological food safety risk assessment. (WHO, 2009).

The responsibility for food safety is given jointly to the Department of Health and the Department of Agriculture (Jackson, 2009). The Directorate for Food Control of the National Department of Health is directly in charge of all matters associated with food safety at national level. Provincial food health control is the responsibility of the nine provincial health authorities (Martens and Anelich, 2000). Some para-statals such as the South African Bureau of Standards are also involved in setting standards for food (Anelich, 2002). Together, these two departments administer 14 different Acts that are related to food issues. These include the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) and the Health Act (Act. The Meat Safety Act (No. 40 of 2000), which was promulgated in November 2000 to replace the Abattoir Hygiene Act (No. 121 of 1992) includes game under the definition of "animal" (Jackson, 2009). Criteria for registration of game farms, culling of game and primary and secondary meat inspection are contained in the Veterinary Procedural Notices (VPN)[1] of the National Department of Agriculture, as listed in Table 1 below.

Table 1: Veterinary Procedural Notices (VPN) applicable to game harvesting [3]

VPN number	VPN name
005 - VPN 05	Standard for the registration or re-registration of game farms for export status
008 - VPN 08	Standard for the registration of hunters for harvesting wild game intended for export of game meat
009 - VPN 09	Standard for the ante and post-mortem meat inspection and hygiene control at point of harvest
010 - VPN 10	Standard for post-mortem meat inspection and hygiene control at game meat establishments
015 - VPN 15	Standard for the microbiological monitoring of meat

Game meat in South Africa has always been available to the select few but this scenario has in the last decade changed due to larger tracts of land now being used as game farms. Usually, game is cropped seasonally during the winter months, when grazing is limited due to the low precipitation and for generation of income (Bothma, 2002). These carcasses are in many cases exported and thus undergo primary and secondary meat inspection. Edible offal, which has already been inspected to certify it disease and pathogen free, currently appears not to be utilized for human consumption (van der Merwe, 2004). Meat by products constitute between 50-60% of the yields of slaughtering, depending upon the species slaughtered (Subba, 2002) . In the game industry, this is sometimes taken to vulture restaurants or left out for predators, after primary meat inspection (Bothma, 2002).

According to the World Trade Organisation (WTO), the importance of risk assessment is as a science-based approach to fair trade (WHO, 2009). Currently, the Codex Alimentarius Commission (CAC), which establishes international food safety standards, is developing principles for the use of risk assessment in establishing such standards. The Joint Expert Meeting on Microbiological Risk Assessment (JEMRA) was established by the Food and Agriculture Organization and the World Health Organisation (WHO), to facilitate the provision of expert advice on microbiological food safety risk assessment. If risks are assessed, managed and communicated in a way that is appropriate to the preconditions in developing countries, substantial capacities in food safety management may be built and evidences of impact may be provided. "Farm to fork approaches" are promising when it is aimed to determine, where risks can be managed most sufficiently (ILRI, 2007; Nguz, 2007) .

Participatory approaches are increasingly recommended as efficient tools when targeting sustainable development (Chambers, 1994; McCrindle, 2003; Van den Hove, 2006). Previous studies dealing with food safety indicated that participatory approaches are very helpful when research is constrained by problems such as data scarcity, an insufficient building of stakeholder ownership and difficulties in ensuring a sustainable risk management. "Participatory Rural Appraisal" (PRA) was developed as an advancement of Rapid Rural Appraisal in the 1990's. (Catley & Mohommed, 1996; Chambers, 1994). Amongst different approaches of risk assessment, participatory risk assessment allows the involvement and the empowerment of participants as well as to rapidly generate reasonable and valid data (Grace *et al*., 2008). It aims to empower communities by giving them the opportunity to actively participate in the definition of problems, finding of solutions, implementation of activities and the evaluation of results obtained from interventions. Participatory risk assessment is a useful tool to better understand the underlying reasons for existing prerequisites and practices. If well conducted participatory risk assessment is very effective in building cooperation and trust amongst stakeholders, roleplayers and end-users that participate (Holloway *et al.*, 2008). Participatory approaches provide an opportunity to build consensus around potential programmes of action through the deliberation by key stakeholder groups (Chadwick *et al.,* 2008). According to Grace *et al.* (2008), risk-based approaches designed to manage food safety in developed countries, need to be modified in order to be applicable to the more challenging conditions of informal markets found in third-world countries. In this context, participatory research is recommended as a "boundary-spanning mechanism", which is able to bring communities and food-safety implementers together. This way, food-safety problems can be analyzed and workable solutions uncovered. Such methodologies helped to develop a new approach to assessing and managing food safety in poor countries which is called 'participatory risk analysis' (Grace *et al.*, 2008).

It is suggested that edible by-products should be utilized efficiently whenever game is harvested (Damm, 2005; Féron et al.,1998; Rao & McGowan,1998; Skinner, 1970). However, little research has been carried out on the potential utilization of legs and offal from

game except for a paper by Van Zyl & Ferreira (2004). The aim of this study was to investigate if edible offal from game culling and trophy hunting could be used to promote food security in rural communities.

METHODS

The study area, Pongola, is in Kwa-Zulu Natal in the Zululand District. It is bordered by Mozambique and Swaziland, with the Indian Ocean to the East and the rest of Kwa-Zulu Natal to the south. Pre-chilled samples of five randomly selected inspected impala carcasses, from each batch sent to Mosstrich Abattoir, were submitted for microbiology to Swift Laboratories, in line with VPN 15 (5 gram incision samples of small game such as impala are similar to those used for sheep). Structured interviews were held with all stakeholders, including veterinarians, game harvesters and informal traders. Scenario planning and decision tree analysis was used to develop a practical food marketing chain and estimate the risk of identified physical, biological and chemical hazards for edible offal marketed through informal markets. In addition, environmental hazards were considered and a risk mitigation strategy designed, to reduce any possible environmental impacts.

RESULTS

Table 2 shows the number of game animal of each species sampled in 2008 at Mosstricht abattoir, which is an export abattoir for game meat. It gives some idea of the relative importance of impala as systematic random sampling is performed on each batch of game that goes through the abattoir.

Table 2: Number of game carcasses of different species sampled in 2008

Blesbok	Blue Wildebeest	Kudu	Impala	Zebra	Date
1	19	34	123	8	26/08/2008
	24	12	72	13	29/08/2008
	19	30	137	9	27/08/2008
	44	6	62	11	02/09/2008
	37	6	12	17	04/09/2008
	46	1	1	20	04/09/2008
	23			10	06/09/2008
	49	5	37		05/09/2008
	14	3	253		10/10
	22	25	172		18/10
	30	11	82	13	22/10
	21	9	116	11	23/10
	10	17	89	8	24/10
		8	259		27/10
	12	26	157		18/10
TOTALS					
1	**370**	**193**	**1765**	**120**	**2449**

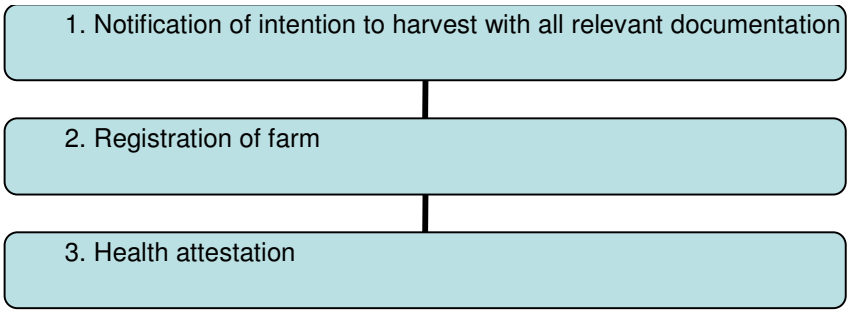
As mentioned in methods, structured and informal interviews were undertaken with all stakeholders in the game meat food value chain in order to identify and characterize potential hazards. Using the data obtained critical control points (CCP) were identified along this food value chain. The risk that these hazards posed at each CCP, was estimated on a qualitative scale and categorized as High, Medium, Low or negligible (none) (Table 3).

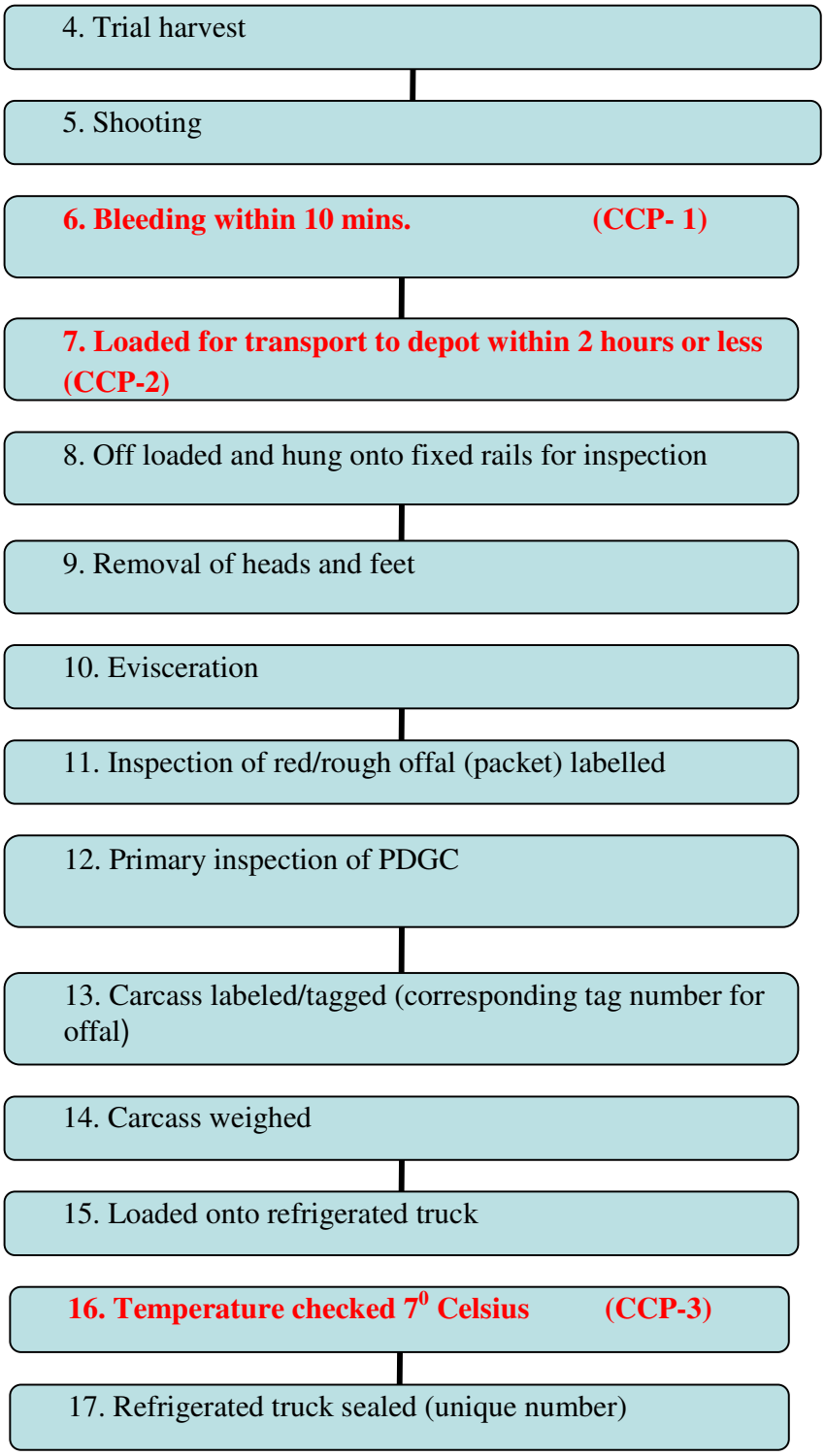
Table 3: Results of participatory risk assessment with all stakeholders: hazard characterization and estimated magnitude

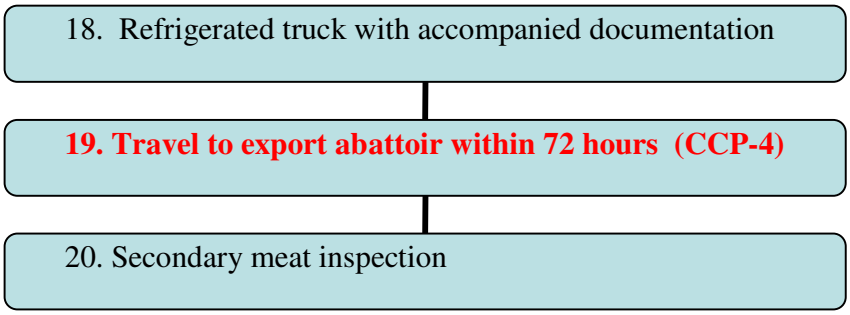
CCP number	Description of hazard	Estimated risk			
		High	**Medium**	**Low**	**None**
1	1.1 Biological hazard: • Increased surface exposed –bacterial contamination • Microbiological count increase 1.2 Physical hazard • Occupational hazard –harvester • Dust,leaves,twigs,grass,stones • Metal filings from knife sharpening 1.3 Animal Welfare Hazard-stressed animal-due to inappropriate shot.	**High** **High** **High**	**Medium** **Medium**		
2	2.1 Biological hazard: • Bloating due to delayed evisceration • Carcass condemned-high risk of bacteria 2.2 Physical hazard: • Dust,stones,leaves,metal filings 2.3 Chemical hazard: • Chemical spillage –alteration of taste	**High** **High** **High**		**Low**	
3	3.1 Biological hazard: • Temperature control-increase bacterial growth if fluctuations occur	**High**			
4	4.1 Biological hazard:spoilage bacteria->72hours	**High**			

Specimens were taken for microbiology in accordance with the appropriate VPN and the requirements of the European Union for venison or game meat. Figure 1 below shows a Hazard Analysis Critical Control Point (HACCP) flow diagram of the game meat food value chain with appropriate CCP's.

Figure 1: HACCP flow chart for game with critical control points indicating identified hazards




4. Trial harvest
5. Shooting
6. Bleeding within 10 mins. (CCP- 1)
7. Loaded for transport to depot within 2 hours or less (CCP-2)
8. Off loaded and hung onto fixed rails for inspection
9. Removal of heads and feet
10. Evisceration
11. Inspection of red/rough offal (packet) labelled
12. Primary inspection of PDGC
13. Carcass labeled/tagged (corresponding tag number for offal)
14. Carcass weighed
15. Loaded onto refrigerated truck
16. Temperature checked 7^0 Celsius (CCP-3)
17. Refrigerated truck sealed (unique number)



During investigation of the food supply chain for game meat, certain environmental hazards, impacts and consequences were observed and mitigation strategies proposed. These are summarized in Table 4.

Table 4: Observed environmental hazards and mitigation strategies suggested

Environmental hazards	Mitigation strategies
1. Biological waste : aesthetic condemnations	Vulture restaurants, predators
2. Biological waste: biohazardous condemnations	Burial in line with VPN
3. Ingesta inside rumen and intestines	Collected and sent to vulture restaurants
4. Contaminated effluent (water)	Correct drainage avoid water table contamination
5. Contaminated efffluent (blood)	Collected on drip trays to avoid environmental contamination.

Biological waste condemned for aesthetic reasons, includes rough offal, heads, feet and trimmings. This aesthetic condemned material is sent to vulture restaurants, or is left out for the predators on game farms, or in some instances it is sent to crocodile farms. Condemnations of bio-hazardous materials, which are septic condemned material, are buried in compliance with the promulgated guidelines of the appropriate Veterinary Procedural Notices (VPN/09/2003-05) for red meat.

The ingesta within the rumen and intestines is also collected, mixed and sent to vulture restaurants. This could, in future, very easily be used for composting or else spread on different parts of the game reserve for degradation through natural processes such as insects and bacterial decomposition. Effluents from game abattoirs or on farm slaughter and primary meat inspection could be contaminated with chemicals used for sterilization of knives or cleaning of transport vehicles. Effluent management should always be factored into a holistic approach to the utilization of game meat. Incorrect drainage can sometimes interfere with the water table and this should be avoided at all costs.

During initial dressing and primary meat inspection in the field, blood is collected on stainless steel drip trays to avoid environmental contamination. This method assists in the control and reduction of the fly population in the field.

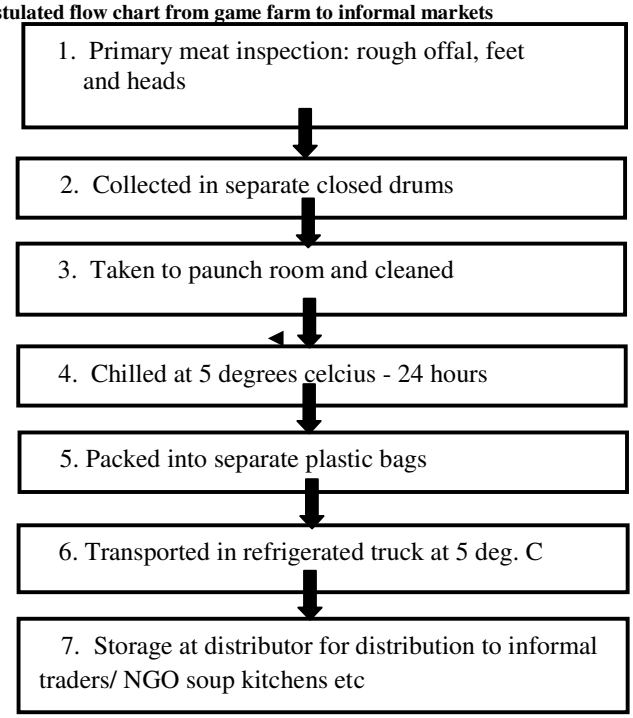
The value of game meat as a nutritional supplement to promote food security is emphasized by the data in Table 5, where the nutritional value is summarized. It can be seen that fat is lower and protein is higher than that found in meat of domestic animals. This is a further motivation for the use of edible game byproducts such as the heads and feet, as an affordable source of food for low income rural communities, particularly those that are most vulnerable such as children, the elderly and those that are immuno-deficient.

Table 5: Nutritional Value of Game Meat

Species	Fat Buttock	Protein Carcass	Cholestrol	Moisture	Calories
	(g/100g)	(g/100g)	mg/100g	(g/100g)	
Gnu	2.30	20.20	46.05	77.00	
Springbuck	1.70	23.70	62.00	74.70	130
Blesbok	1.70	23.50	51.38	75.50	
Kudu	1.58	21.50			
Impala	1.40	22.50		75.70	
Eland	2.40			75.80	
Gemsbok	1.90			75.90	
Hartebees	2.00			76.30	
Mutton	21.60	13.90	75(1)	60.70	205
Ostrich	3.00	21.10	58	76.30	97
Pork	17.60	13.90	93(1)	55.00	275
Beef	14.20	19.20	99(1)		240
Chicken			81		140

This study has suggested that edible offal could be marketed to the poor. Currently there is a thriving informal market that supplies food to taxi commuters and school children. Thus the same participatory methods were used to postulate a new food value chain from primary meat inspection of game meat on farm, to the low income consumer via informal markets. Table 6 shows the food value chain developed form the data obtained in informal markets and applied theoretically to the marketing of edible offal.

Table 6: Postulated flow chart from game farm to informal markets



DISCUSSION

There is an abundant seasonal supply of game by products with offal being the largest source of protein that is currently being condemned as per VPN regulations. This offal and other game by-products can become part of the food value chain for the informal markets, if the above flow chart was adopted to include some basic prerequisites so that food safety and quality is not compromised.
The above chart was put together by a team that was inclusive of the informal traders, since their feed-back of first hand experiences at the ground level identified the needs of their clients. The proposed prerequisites should be based on hygiene norms suggested by health inspectors in line with internationally recognized norms for informal street vended food, such as a basic hand washing plan, together with a programme that emphasizes the cold chain and handling of any food products of a highly perishable nature. Due to its highly perishable nature, game offal should first be transported in closed plastic drums from the field to the paunch room on the game farm. Here it should be cleaned with running water. Once the offal is cleaned it is proposed that it should be packed into plastic bags and is then put into a chiller with a temperature of 5 degrees Celcius and kept overnight. The maintenance of the cold chain would allow for the product to be safely distributed to more remote rural areas.
A refrigerated (0-5 degrees) transport truck could collect the chilled offal and take it to a central distributor where it could be frozen and stored. The frozen product could either be sold to informal traders, or donated to soup kitchens or schools in low income areas. It could even be used to make traditional foods, such as tripe, for restaurants catering to the tourist trade. These options would promote job creation for women and youth as well as improving food security in remote rural communities near to game farms and parks. This could thus become a sustainable programme, although there is currently a seasonal over-supply of game meat and by-products, these could be frozen for use at a later stage.
Additional training in environmental awareness, effluent and waste management, food handling, food borne diseases, personal hygiene, and product handling are areas that need to be addressed as a matter of urgency to ensure food safety for edible offal from game meat.

CONCLUSIONS

It was concluded that the food marketing chain for edible by-products of game slaughter, is feasible and that the current practice of leaving the offal for predators was likely, if it continues, to cause imbalances in the predator/ prey ratio on game farms.

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CLASSICAL SWINE FEVER CONTROL IN SOUTH AFRICA 2008-09: RESULTS OF THE DISEASE SURVEILLANCE IN THE EASTERN CAPE PROVINCE

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SUMMARY

The efforts to control Classical Swine Fever (CSF) which last occurred in South Africa in 1918 but re-entered the country with devastating consequences during 2004 seem to have succeeded. The disease which was confirmed in pig serum and tissue samples from the Eastern Cape (2005) and Western Cape (2004) provinces of South Africa has not been detected in the Eastern Cape Province since October 2007.

CSF affected large parts of the Eastern Cape Province that practice the communal system of livestock keeping between 2005 and 2007. This open type of farming system could not be easily delineated into conventional epidemiological zones for surveillance, quarantine or control purposes. In order to control CSF, an approach involving total depopulation of pigs from the area of coalescence of all identified foci positive for CSF virus (CSFV) was adopted. This culminated in the culling of 493000 pigs, mostly from the communal farming areas of the Eastern Cape Province to the north of the Kei River from August 2005 until the conclusion of the CSF eradication campaign in April 2007. No more CSFV positive foci were detected until August and October 2007 respectively. The 2 foci were located within communal areas that were not part of the depopulation exercise and were culled out promptly. Serological surveillance for the disease was continued thereafter and by December 2008 no more foci positive for CSFV infection were found.

A final endeavour to conduct an intensive serological surveillance of pigs throughout the Eastern Cape Province was undertaken between June and September 2009, where 8427 sera samples were collected from more than six hundred different points within the province. Most of the samples originated from areas where pigs had previously not been culled out completely. However, a significant number of samples were also collected from areas where pigs were expected to have been culled out completely during the CSF eradication campaign. Pigs were present in the culled areas despite no official restocking of these areas having taken place. All samples including those collected from these areas tested negative for CSF. The same samples, where possible, were also tested for Porcine Respiratory and Reproductive Syndrome and for Swine Influenza and all were found to be negative for those diseases as well. These findings might indicate that CSF has been controlled if not eradicated from the Eastern Cape Province.

KEY WORDS

CSF, pigs, eradication, communal grazing

INTRODUCTION

Classical Swine Fever (CSF) (Hog Cholera or European Swine Fever) is a haemorrhagic, immunosuppressive and reproductive failure causing disease of domestic pigs and wild boars mainly affecting North America and Europe (Moennig, Floegel-Niesmann & Greiser-Wilke, 2003). The causative organism, classical swine fever virus (CSFV), can be transmitted by various direct and indirect methods. Infection with CSFV must be confirmed in the laboratory where serological and antigen identification test methods can be employed to confirm diagnosis. The disease was last reported in South Africa in 1918 (Plowright, Thomson & Neser, 1994), but re-emerged during 2004 in the Western Cape Province and spread to the Eastern Cape province most likely in 2005 (Sandvik, Crooke, Drew, Blome, Greiser-Wilke, Moennig, Gous, Gers, Kitching, Bührmann, & Brückner, 2005; Akol, Rozani, Gerdes, Vosloo, Lubisi, Botha, Dwarka, Drew, Zondi, Mrwebi, & Lwanga-Iga, 2006). Since South Africa was not endemic for CSF, the option of stamping out the infected and in-contact suids was opted for over vaccination, which is mainly practiced in endemic countries for outbreak control. Unlike the $2.3 billion costs incurred from the stamping out of pigs in the 1997 and 1998 outbreak control and eradication attempt (Elbers, Stegeman, Moser, Ekker, Smak, & Pluimers, 1999), the cost of the apparent 'eradication' of the South African CSF epidemic is unknown. While the cost to the state in terms of controlling the disease was estimated at over $100 million, the additional cost in terms of loss of income especially for livelihoods of communal farmers in rural areas was probably greater (Akol, 2008). No outbreak of CSF was detected in the Province since the last confirmed case in October 2007. In order to provide appropriate data for a final evaluation of the CSF disease status in the Eastern Cape Province and country, an intensive 3 month serological surveillance campaign was conducted in the province between June and September 2009, as part of a national serological survey targeting 7 diseases affecting swine. This paper describes results of the surveillance with special focus on the Eastern Cape Province.

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MATERIALS AND METHODS

STUDY AREA

The Eastern Cape Province is 168,966 km2 in size and situated in the South Eastern Coast within the Republic of South Africa. The province consists of 6 districts, which were all sampled during the surveillance exercises, including the supposed pig 'depopulated' areas (Fig. 1). Commercial and non-commercial holdings practicing embracing different pig keeping methods and feeding practices such as self mixed or swill feeding, and those rearing their pigs outdoors were targeted.

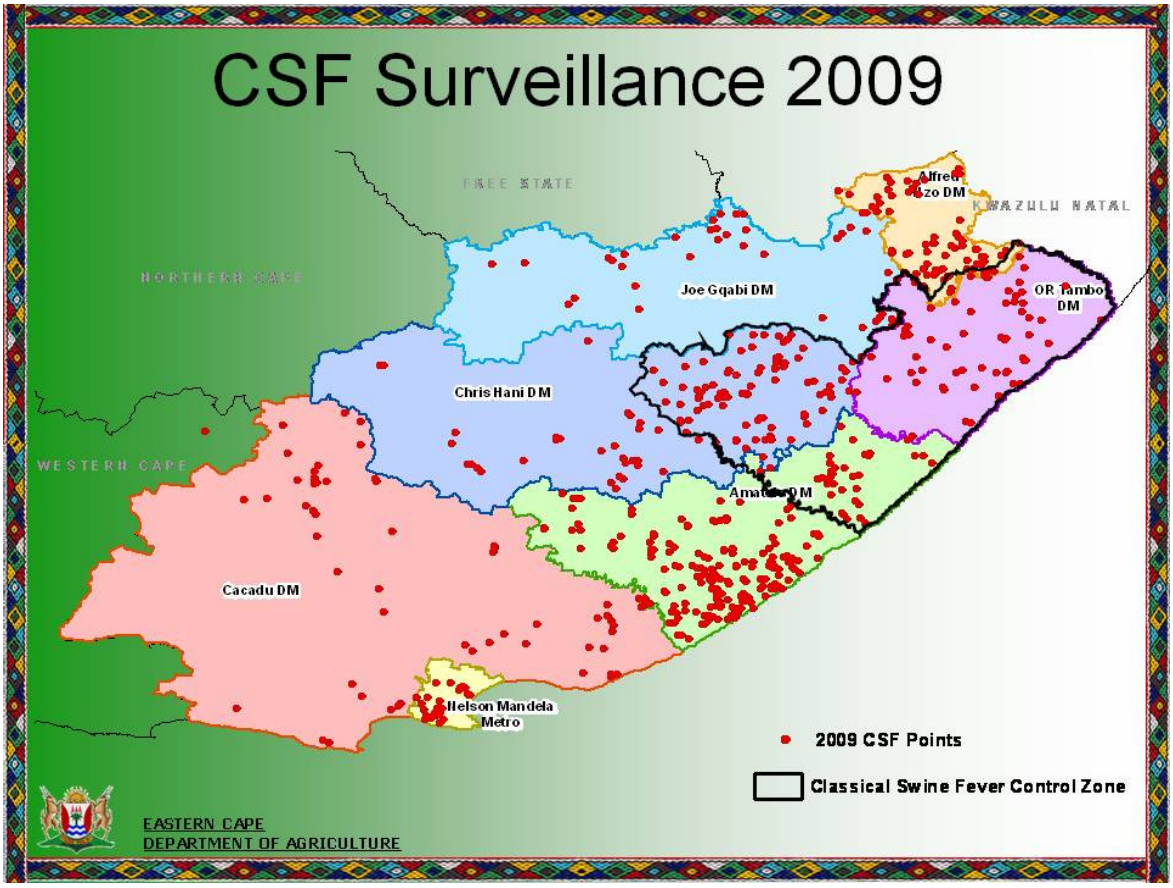

Figure 1: Map of the Eastern Cape Province showing the six district municipalities (colour coded) and the CSF controlled area delineated in black. Spatial distribution of the sampling points for CSF in the Eastern Cape Province during 2009 is represented by the red spots

SAMPLING STRATEGY

For routine CSF surveillance conducted between 2005 and June 2009, samples were collected using a specific protocol designed by both the national and provincial veterinary authorities, and up to a maximum of 35 blood samples were to be collected per epidemiological unit for serological testing. In communal areas an entire village was considered a single epidemiological unit. In cases of pig mortality suspected to be due to CSF, fresh tissue specimens (tonsil, spleen, kidney, mesenteric & maxillary or sub-mandibular lymph nodes and distal ilium) were collected and transported on ice to the laboratory for CSFV antigen detection.

The June 2009 to March 2010 national pig serological survey comprised at least 18000 sera constituted by 15 600 and 2400 non-commercial and commercial specimens respectively. Sixty sampling points were chosen for the Eastern Cape Province, and 20 samples per sampling point were aimed for, thus at least 1200 of the survey's 18000 sera were to originate from the province. However, samples collected shortly prior to the official commencement of the surveillance and those obtained to exceed the required

numbers per sampling point were incorporated in the survey, bringing the total number of samples tested for CSF from the province to 8427 from at least six hundred points. Essential data such as province, sender, GIS location; and OVI reference number were captured per sampling point.

Provision was made to perform clinical and post mortem examinations on sick but live, and dead pigs respectively, and collect additional samples for diagnostic purposes if CSF was suspected.

LABORATORY ASSAYS

All laboratory tests were conducted at Agricultural Research Council – Onderstepoort Veterinary Institute (ARC-OVI). The 2009 to 2010 national pig survey was conducted in two phases, where testing for classical swine fever (CSF), porcine reproductive and respiratory syndrome (PRRS) and swine influenza (SI) was performed in all 18000 plus samples in the first stage in 2009; and screening for pseudo rabies (PR), transmissible gastroenteritis (TGE), foot and mouth disease (FMD) and African swine fever (ASF) was executed in 6000 sera in the second stage in 2010.

SEROLOGY

The commercial CSF antibody blocking ELISA kit - HERDCHECK*CSFV Ab (IDEXX Europe B.V., Koolhovenlaan 20 Schiphol-Rijk, Netherlands) was employed for screening sera for anti-CSFV antibodies.

In brief, reagents were equilibrated to room temperature (18^0C to 25^0C) and 50 µl of sample diluent added to all wells of the 96 well microtitre plate. The same volume of control and test sera were added to the appropriate wells, reagent mixing was achieved by gentle tapping of the plate. The plates were incubated in a humid chamber at room temperature (18^0C to 25^0C) for 2 hours or at room temperature overnight. Following incubation, each plate was washed three times with approximately 300µl of wash solution per well, ensuring proper aspiration and avoiding drying. Hundred micro litres of anti-CSFV/Horseradish Peroxidase (HRPO) conjugate was added to each well and 30 minutes incubation at room temperature in a humid chamber was allowed. Washing as described above was performed, and 100µl of TMB substrate solution was added. Following a 10 minute incubation period at room temperature in darkness, the reaction was stopped by adding 100µl of Stop Solution to each well. The absorbance of the samples and controls was measured at 450nm and 650nm on a microplate reader. A test sample was regarded as positive for anti-CSFV antibodies if it gave a blocking percentage equal or greater than 40%, and negative if the resultant blocking percentage was equal to or less than 30%. Blocking percentages between 30 and 40% were regarded as doubtful.

ANTIGEN DETECTION

The anti -E2 viral protein antibody based antigen-capture ELISA -Herdchek* CSFV Antigen Test Kit (IDEXX Europe B.V., Koolhovenlaan 20 Schiphol-Rijk, Netherlands), was utilized for CSFV antigen detection in porcine peripheral blood leukocytes, whole blood and organs as per manufacturer's instructions. PCR analysis was performed as described in a previous paper (Akol *et al*., 2006).

RESULTS

A total of 95291 samples consisting of sera and tissues from pigs were tested for CSFV antibodies and antigen accordingly in the Eastern Cape Province between August 2005 and December 2009; of which 74449 originated from communal areas and 20842 were obtained from commercial farms and small holdings. Between 2005 and 2007, 1913 samples from communal areas and 197 from commercial farms were positive for either CSFV antigen or antibodies against the virus (Table 1).

Table 1: Sampling indices and combined CSFV antigen and antibody screening results of Eastern Cape pigs from 2005 to 2009

	Samples Tested		CSF Positive samples			
	Communal	Commercial	Communal		Commercial	
			Number	Percentage	Number	Percentage
2005	20921	4413	1224	5.85%	132	2.99%
2006	25865	5575	494	1.91%	25	0.45%
2007	13858	6151	195	1.41%	40	0.65%
2008	5700	2750	0	0.00%	0	0.00%
2009	7390	2668	0	0.00%	0	0.00%
Subtotal	74294	20997	1913	2.57%	197	0.94%
Total	95291		2110 (2.21%)			

Following the termination of the pig culling campaign on 30th April 2007, an infected foci was discovered in August 2007 in Machibi village (27 26 41E; 33 00 48 S), Peddie state veterinary (SV) area, Amatole district municipality. Another infected location was detected in October 2007 in Mnxekazi administrative area (29 02 30E; 31 01 39S), Ntabankulu, Qumbu SV area, OR Tambo district municipality. A few tissues (<5%) harvested from dead or culled pigs and sera collected from pigs within the two infected villages, tested positive for CSFV antigen or antibody. PCR tests for tissue samples from Mnxekazi village were mostly inconclusive. All 563 pigs in both newly discovered infected and surrounding villages were culled, bringing the total number of pigs culled in the province as a result of CSF control since the initial outbreak in 2005 to 493086.

Clinical signs of CSF such as loss of condition, weakness and staggering gait and necropsy lesions characterised by inflamed oedematous petechiated or necrotic tonsils were seen in some of the pigs culled in Machibi village. No clinical evidence of disease or lesions suggestive of CSF were observed in Mnxekazi village. Blood and tissue samples for viral antibodies and antigen detection respectively, were obtained from 65 and 17 pigs during the culling process at Machibi village accordingly. Only 1 of the 17 tissue samples tested positive for CSFV antigen, and 26 of the 65 sera yielded positive results for antibody presence.

By December 2007, 20009 sera and tissue samples were tested for CSFV antibody or antigen, and 235 of which yielded positive results (Table 1). All other positive reactors besides those from the 2 disease foci in Peddie and Qumbu SV areas were regarded as non specific serological reactors. The antibody positive reactors occurred in various parts of the province. No evidence of clinical infection was obtained nor positive laboratory results acquired during follow up investigations in the areas yielding non specific reactors. The areas were however routinely sampled and tested and found to be negative for CSF.

Serological surveillance of pigs for CSF continued during 2008 and 2009 nonetheless. Most of the surveillance sera samples originated from Alfred Nzo district municipality which was not depopulated of pigs during the 2005 to 2007 CSF eradication campaign (Table 2). The sera samples collected from Western district municipality consisted primarily of material sourced from abattoirs. Significant numbers of samples were also collected from OR Tambo which had been depopulated completely except for Ntabankulu local municipality. All samples tested negative for CSFV antibody or antigen. The last CSF positive cases were diagnosed in 2007.

Table 2: District sample contribution to the CSF antibody and antigen surveillance conducted in the Eastern Cape between January 2008 and December 2009. No positive results were obtained for the period

District Municipality	Total samples tested[1]	% of the provincial totals
Joe Gqabi (Ukhahlamba)	1992	10.76
Alfred Nzo	4262	23.03
Western (Cacadu & NMMM)	4101	22.16
Chris Hani	1423	7.69
OR Tambo	3007	16.25
Amatole	3723	20.12
Provincial Total	**18508**	**100.00**

NMMM – Nelson Mandela Metropole Municipality

DISCUSSION

All pigs within and around the CSFV infected foci of the Eastern Cape Province were believed to be culled out by April 2007, as described previously (Akol and Mrwebi, 2008). However, post culling sero-surveillance revealed 2 infected locations in Peddie and Qumbu SV areas respectively. The source of infection in the two areas was uncertain. It is likely that the newly discovered infected areas represented hidden infection foci that were not detected during the major surveillance campaign for the disease. This could be the case especially for Machibi village which is located on the border between Zwelitsha and Peddie SV areas. Mnxekazi village in Qumbu SV area is also fairly isolated. Furthermore, the initial indication of the presence of CSF at Machibi village was recognized by the SV Zwelitsha since the SV Peddie position was vacant at the time. In addition, the clinical signs observed were of a chronic nature and some of the relatively high CSFV Elisa antibody titers reflected an infection that may have been in the area for a long time.

The disease recrudescence in these areas of the Eastern Cape could probably be linked to failure to achieve the improbable target of a 100% cull rate under the free ranging pig farming system in communal grazing areas. The presence of an inhospitable terrain, poor community cooperation associated with the slow compensation of pig owners by the government, as well as unknown factors such as the disease status in wild pigs (Akol and Mrwebi, 2008) could all contribute to reoccurrence of the disease. These factors combined, could make eradication of CSF difficult if not impossible under free range conditions. Nonetheless serological surveillance during 2008 and 2009 where 18508 sera were tested did not reveal any pig infection with CSFV in the Eastern Cape Province.

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VACCINATION OF DOMESTIC PIGS WITH A NATURALLY ATTENUATED STRAIN OF AFRICAN SWINE FEVER VIRUS

P. Mutowembwa[1], R. Souto, J. van Heerden & L. Heath

INTRODUCTION

African swine fever (ASF) is an acute haemorrhagic fever in domestic pigs which causes mortality approaching 100%, depending on the strain involved. It causes large economic losses to the rural and peri-urban poor pig farmers as well as commercial farmers. In areas were ASF is endemic the disease impacts negatively on the sustainability of farming practices thereby limiting the availability of cheap, high-quality proteins and steady income streams for rural communities.

Control measures are largely based on movement restrictions and stamping out in case of an outbreak. There is currently no vaccine available for ASF. However, pigs immunised with live attenuated ASF viruses, containing engineered deletions of specific ASFV virulence/host range genes, were protected when challenged with homologous parental virus (Lewis et al., 2000). An analogous situation has been reported in parts of southern Africa where apparent resistance to the disease in domestic pigs has been observed in several endemic regions (Bastos, A. D. S., Penrith, M- L., Macome, F., Pinto, F. & Thomson, G. R. (2004); Penrith, M. L., Thomson, G. R., Bastos, A. D. S., Phiri, O. C., Lubisi, B. A., Du Plessis, E. C., Macome, F., Botha, B. & Esterhuysen, J . (2004)). It is suggested that the resistance could possibly be contributed to the repeated exposure of animals to the virus with reduced virulence, resulting in the development of acquired immunity.

In order to identify strains that could potentially be used as an attenuated vaccine, the pathology caused by an ASFV strain typically found in southern Africa was compared to strains originating from West Africa and Europe. As a second objective the ability of a fully attenuated European strain to protect pigs against challenge with a highly virulent African strain was investigated.

MATERIALS AND METHODS

ASF VIRUSES

Two isolates representative of strains circulating in southern Africa were selected for inclusion in the study. The first, a non-haemadsorbing ASF virus (Mkuze /78) was isolated from an *O. procinus* tick collected from a warthog burrow in the Mkuze Game reserve, South Africa (Thomson, G. R. (1985)). The second African strain used in the study, Moz 1/98, was isolated from a domestic pig that died of ASF during translocation from Mozambique to South Africa (Penrith, M. L., Thomson, G. R., Bastos, A. D. S., Phiri, O. C., Lubisi, B. A., Du Plessis, E. C., Macome, F., Botha, B. & Esterhuysen, J. (2004)).

The reference viruses used were a highly virulent isolate (Ben 1/97) isolated from a domestic pig in Benin (Chapman, D. A. G., Tcherepanov, V., Upton, C. & Dixon, L. K. (2008)) and an avirulent isolate from a *O. erraticus* tick (OURT 3/88) collected in the Alentejo province in Portugal (Boinas, F. (1995)).

INFECTION OF PIGS

Four groups of 3 cross-bred Large White/Landrace pigs of 20–30 kg (live weight) were infected intramuscularly with 10^4 $TCID_{50}$ ASFV prepared in pig bone marrow (PBM) cells. Clinical examination and rectal temperatures were recorded each day. Viraemia was monitored at 1, 3, 5, 7, 14 and 21 days post-infection (dpi). Serological responses were monitored on a weekly basis. This was carried out until the animals died or were euthanized 3 weeks post infection. Detailed necropsies were performed on each pig included in the study.

VACCINATION OF PIGS

Two groups of 6 pigs were vaccinated with 10^4 $TCID_{50}$ of the live avirulent OURT3/88 isolate. One group received a second vaccination using the same virus 3 weeks after the first vaccination. The groups were subsequently challenged with 10^4 $TCID_{50}$ virulent Moz1/98. Clinical examination and rectal temperatures were recorded each day. Viraemia was monitored at 1, 3, 5, 7, 14 and 21 days post-vaccination and at similar intervals following challenge. Serological responses were monitored on a weekly basis. This

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was carried out until the animals died or were euthanized 3 weeks post infection. Detailed necropsies were performed on each pig included in the study.

RESULTS

INFECTION OF PIGS

In a previous research study, a strain of the virus was isolated from soft ticks originating from the Mkuze game reserve in the KwaZulu-Natal province of South Africa. Phenotypic characterization of the isolate *in vitro* revealed that it has lost the ability to bind red blood cells. The inability of this isolate to bind red blood cells is due to the deletion of the gene encoding the CD2v protein. Deletion of this gene from the genome of a virulent HAD have been shown to delay the onset of viraemia and dissemination of viruses within infected pigs (Borca, M. V., Carrillo, C., Zsak, L., Laegreid, W. W., Kutish, G. F., Neilan, J. G., Burrage, T. G. & Rock, D. L. (1998)) and has previously been link to natural attenuation of ASFV.

A controlled infection experiment to gauge its level of virulence was conducted to determine natural attenuation of this virus. Results suggested that this strain of the virus has a reduced virulence in domestic pigs. The onset of clinical signs was delayed by 6 days with low levels of viraemia detectable only 19 days post inoculation (Table 1). Two out of the three pigs inoculated develop antibodies against ASF eight dpi (Table 1). Results suggest that although an ASFV strain isolated from ticks in KwaZulu-Natal displayed reduced pathogenesis, it is not sufficiently attenuated to be used as a live vaccine.

A strain of ASF isolated in 1998 from domestic pig from Mozambique was included in the experiment as representative of viruses commonly implicated in sporadic outbreaks throughout southern Africa. The Mozambican strain exhibited high virulence consistent with clinical finding reported during outbreaks of the disease in the region. Al three pigs developed clinical signs of disease within 3 dpi and died 5 days later (Table 1).

Table 1: Characteristics of disease caused in pigs by ASF strains isolated from ticks and domestic pigs

Strain	Days post infection			
	Clinical Signs	**Viraemia**	**Sero-conversion**	**Death**
OURT 3/88	Never	Never	8	20*
BENIN 1/97	3	4	Never	8
MKUZE /78	9	19	8	19
MOZ 1/98	3	4	Never	8

***These animals were euthanized at the end of the trial**

VACCINATION OF PIGS

As a second objective the ability of a fully attenuated European strain to protect pigs against challenge with a highly virulent African strain was investigated. Groups of six pigs were vaccinated either once or twice with OURT 3/88 and subsequently challenged with the virulent Moz 1/98 strain. None of the pigs vaccinated with the attenuated stain developed clinical signs or viraemia, but test positive for antibodies to ASF at 7 dpi (Table 2). Despite the presence of antibodies both single and multiple vaccinations regimes failed to confer protection against subsequent challenge. All vaccinated pigs presented with clinical signs at 3 dpi (Table 2). Viraemia was detectable 3 dpi (Table 2). All pigs succumbed to the infection within 11 dpi (Table 2).

Table 2: Characteristics of disease caused in pigs vaccinated with a live attenuated strain following challenge with virulent ASFV.

Group	Days post challenge			
	Clinical Signs	**Viraemia**	**Sero-conversion**	**Death**
Single vaccination (OURT 3/88)	3	3	N/A	5-9
Boost Vaccination (OURT 3/88)	3	3	N/A	5-11
MOZ 1/98	3	3	N/A	4-5

DISCUSSION

Although vaccination of susceptible pigs did briefly delay the initial onset of disease, it had no effect on disease development, progression or outcome. It should be noted though that the strain used here was isolated from ticks in Europe and is genetically distinct from strains typically circulating in southern Africa. It has been shown that infection with attenuated strains confers protection to pigs against homologous challenge and therefore it is conceivable that partial protection could be attained following vaccination with a more attenuated African virus. However, the results described here is consistent with previous reports suggesting that in some cases, European ASFV isolates may be more attenuated and adapted for domestic pigs than African field strains (Mebus, C. A. (1998); Ordas-Alvarez, A. & Marcotegui, M. A. (1987)). In light of this, efforts to identify naturally attenuated African strains should continue. Alternatively, attenuated viruses based on Africans strains could potentially be developed *in vitro* by site directed mutagenesis or deletion of immunomodulatory genes.

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EPIDEMIOLOGICAL STUDY OF VARROASIS IN HONEY BEE IN GOLESTAN PROVINCE, IRAN

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ABSTRACT

Background and Objectives: The new, worldwide and hemophagous honey bee mite Varroa destructor is considered a main pest of honey bees Apis mellifera. The disease distribution and predisposing the infested bees to other diseases lead to high economic losses in beekeeping industries, and in addition Varroa mite may intensify the problems of pollination in future time. The rapid spread of varroa mites among bee colonies is due to a number of factors such as drifting of diseased bees, movement of bee swarms, robbing of weakened colonies, migration and importation of infested bees. The aims of this study were to determine the prevalence of varroasis and evaluate some managing factors in Golestan province with a temperate and wet macro climate that is one of the most important sites of beekeeping in Iran in 2008.
Method: According to the agricultural research center records 80 beekeeping centers were identified and a questionnaire consisting of managing factors such as literacy and experience levels of bee keepers as well as factors related to apiculture center and hives position was filled for each apiculture center. Varroa mite infestation was confirmed by agricultural research center. All data were recorded and analyzed by SPSS software to calculate χ^2 and fishers exact test.
Results: Among 80 apiculture centers 72 centers (92%) were infested with varroa. Height of hives from the ground in 69 (89.6%) of infested centers was 10-25 cm (P=0.0284), nineteen (79.2%) of infested centers had used a guard wall around the apiculture center (P=0.0485). Additional pollen had consumed in 62(89.8%) positive beekeeping centers (P=0.00011) and river was the water supplying way in 63(88.7%) of affected centers (P=0.0008).
Conclusion: According to the results this high prevalence of infestation shows the high spread of V. destructor in beekeeping centers in Golestan province. In this study we find a significant relation between disease and water supplying system, height of hives, use of additional pollen and use of guard wall. As an external and obligate parasite of honey bees the environmental circumstance for Varroa mites is supply by the bee and beehive. The microclimatic conditions inside the colony are affected by outside factors like temperature, wetness or the accessibility of pollen and nectar. Totally we can conclude that because of the high prevalence of the disease many usual methods of prevention are not effective however this high prevalence emphasizes that we are still far from a solution for varroa infestation and extra researches on mite biology, tolerance breeding, and Varroa treatment is immediately needed.

KEYWORDS

Apis mellifera, Varroa destructor, Prevalence

INTRODUCTION

The new, worldwide and hemophagous honey bee mite Varroa destructor is considered a main pest of honey bees Apis mellifera. Before year 2000 V. destructor was understood to be Varroa jacobsoni (Anderson and Trueman, 2000) which successfully shifted from the original host, A. cerana to the Western honey bee, A. mellifera. (Rath, 1999). The details of the host shift are unclear. V. destructor act as a vector for different bee viruses. Until now 18 different viruses have been identified in honey bees (Chen and Siede, 2007) and numerous of bee viruses can be transmitted by V. destructor for example Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), Sacbrood virus (SBV), and Deformed wing virus (DWV) (Boecking and Genersch, 2008). Before the appearance of Varroa mites in A. melifera, viral diseases were minor troubles for honey bee health (Allen et al., 1986; Bailey and Ball, 1991; Bowen-Walker et al., 1999; Yue and Genersch, 2005). At a short time V. destructor has worldwide geographical distribution and we can not find a country free of this disease other than Australia. The economic burden of this ectoparasite is high, disease distribution and predisposing the infested bees to other diseases lead to high economic losses in beekeeping industries in addition Varroa mite may intensify the problems of pollination in future time (De la Rua et al., 2009).
Varroa females start to reproduction by entering the brood cells of last-stage worker or drone larvae, normally within 20–40 hours before the cells are sealed (Boot et al., 1992). About 60 hours after the bee cells are capped, the adult female mites put their first eggs and each mite can produce over 10 progeny (Sammataro et al., 2000) The adult female mite and progeny feed on the hemolymph of pupae from a single feeding site (Kanbar and Engels, 2003). All reproduction of varroa occurs in the brood cells, and only the adult females survive after the bee emerges. Some immature females, eggs (rarely) and males are left and removed by the nurse bees when the bee emerges. Varroa mites suck the hemolymph from adults and developing pupae of honey bees, thereby weakening the bees and

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reduction their life length. The rapid spread of varroa mites among bee colonies is due to a number of factors such as drifting of diseased bees, movement of bee swarms, and robbing of weakened colonies (de Jong, 1997). In addition, migratory beekeeping practices and the importation of infested bees lead to rapid distribution of varroa mites (Sammataro et al., 2000). Application of synthetic acaricides has been the main way for controlling the pest. But the intensive use of many chemical substances against the mites resulted in the increase of resistance and decrease of their efficiency (Milani 1999) and contamination of products such as honey and beeswax (Wallner 1999). The problems of chemical acaricides promote the idea of finding new and safer ways of Varroa control. Natural products such as essential oils offer a highly desirable alternative to synthetic products. These substances are used increasingly because they are generally inexpensive and have fewer health hazards to both man and honeybees (Isman 2000). Various alternative ways for managing the mite have been investigated (Imdorf et al. 1995; Fries 1997; Thomas 1997; Calderone 2005). Most of them suggest non-chemical methods for reaching lower occurrence of mite infestation in beehives (Imdorf et al. 1999). One of these non-chemical methods is use of formic acid that has received great consideration because of its activity against V. destructor (Calderone 2000; Currie and Gatien 2006).
In Iran based on the veterinary organization protocol beehives with $\leq 5\%$ infestation of V. destructor must be treated with standard treatments such as Formic Acid, Api Life Var, Apiguard, Apistan and Apivar.
The objectives of this study were to determine the prevalence of V. destructor and evaluating the effect of the hygienic factors in infestation rate in Golestan province one of Northern provinces in I.R. Iran with temperate and wet macro climate that is one of the most important sites of beekeeping in Iran in 2008.

METHOD

According to records of the agricultural research center all the beekeeping centers were identified in Golestan province. Varroa mite infestation was confirmed by the agricultural research center according to the veterinary organization protocol. Varroa sampling was done every season and 5% of hives in each beekeeping center were investigated. Beehives with less than 5 honey-combs were excluded from sampling. A questionnaire consisting of managing and hygienic factors were filled for each beekeeping center. Questions were such as distance to the nearest apiculture center, altitude of apiculture center from the sea level, use of guard wall around the beekeeping center, migration situation, number of hives, the height of hives from the ground, distance between hives, disinfection methods, ways to provision of pollen, wax, apiculture equipments, queen and water supply. Literacy and experience levels of bee keepers were also asked. All data were recorded and analyzed by SPSS software version 15 to calculate $\chi 2$ test and fisher exact test. P values less than 0.05 were considered as significant level.

RESULTS

Among 80 apiculture centers 72 centers, 92% were infested by varroa mite. In affected centers, hive density of 90.6% centers was 31-60 hives/center (P=0.324), All of the apiculture centers (100%) had more than 6 Km distance to nearest beekeeping center (P=0.687). Fire was used to disinfect the equipments in 54(90%) affected centers (P=1.000). Fifty eight infected centers (90.6%) had migrated the bees (P=0.657).
Amongst beekeepers 15(93.8%) had low literacy level (P=0.479) and 26 (89.7%) had 5-10 years experience in beekeeping (P=0.953).
In table 1 the association between varroa infestation of beekeeping centers and the altitude of apiculture center from the sea level, the height of beehives from the ground, distance between beehives and use of guard wall around the beekeeping center, are shown.
The ways of provision of pollen, wax, apiculture equipments, queen and water supply are shown in table 2 and their relations with varroasis are shown in same table.

DISCUSSION

According to the results this high prevalence of infestation shows the high spread of V. destructor in beekeeping centers in Golestan province that is one of Northern provinces of Iran with temperate and high humidity climate. This occurrence is similar to other sites of the world that had reported, the eastern coastal region of the USSR in 1952, Pakistan in 1955, Japan in 1958, China in 1959, Bulgaria in 1967, Paraguay in 1971, Germany in 1977 (Ruttner and Ritter, 1980), United States in 1987 (De Guzman and Rinderer, 1999). Today, V. destructor is a global distribution, but according to the reports published by Australian Government at http://www.daff.gov.au/ qis/quarantine/pests-diseases/honeybees it has not yet been found in Australia.
In this study we can show that heights of hive from the ground level associate to varroais prevalence. Distance to the soil can influence the humidity of brood cells and influence the mite reproduction. We found a significant relationship between varroasis and use of additional pollen for hives in beekeeping centers also we can show that infestation rate of beekeeping centers that supply their water from the river are less than centers which provide water from other routes. Also the use of a guard wall around the apiculture center was related to lower infestation rate of varroa. As an external and obligate parasite of honey bees the environmental circumstance for

Varroa mites is supply by the bee as a host and this environmental condition influences by situation within the honey beehive. The microclimatic conditions inside the colony are affected by outside factors like temperature, wetness or the accessibility of pollen and nectar. This may, most likely indirectly, influence the proportion of non-reproducing mites (Eguaras et al., 1994; Garcia-Fernandez et al., 1995; Kraus and Velthuis, 1997; Moretto et al., 1997).

According to the insignificant results of the effect of beekeepers literacy and experience level in prevention of disease and inefficiency of hygienic factors such as having suitable distance to other beekeeping centers and decreasing hive density in a center (number of hives) and the altitude of apiculture center from the sea level and distance between beehives, we can conclude that because of the high prevalence of the disease usual methods of prevention are not effective also the ways to provision pollen, bee wax, equipments, and queen had not any influence in varroa infestation rate.

Use of varroa tolerant honey bees and chemical and biological methods of varroa treatments are considered as important controlling ways. The control of mite reproduction is considered the most effective tool for the host to prevent the growth of a Varroa population within the colony (Fries et al., 1994).

However this high prevalence highlights that we are still far from a solution for varroa infestation in honey bees and further exploration on mite biology, tolerance breeding, and treatment especially by non-chemical substances is instantly needed.

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Table 1: The relation between varroasis and the altitude of apiculture center, the height of hives, distance between hives and use of guard wall

Variables \ Varroasis		Positive	Negative	p value
Altitude of apiculture center	≤800m	47(90.4%)	5(9.6%)	P=1.000
	>800m	25(89.2%)	3(10.8%)	
Height of hives from the ground	10-25cm	69(89.6%)	8(10.4%)	P=0.0284*
	26-40cm	3(100%)	0(0%)	
Distance between hives	≤50cm	63(90%)	7(10%)	P=1.000
	>50cm	9(90%)	1(10%)	
Use of guard wall	Yes	19(79.2%)	5(20.8%)	P=0.0485*
	No	53(95%)	3(5%)	

***P<0.05 is significant**

Table 2: The relation between varroasis and the use of pollen in apiculture, source of pollen, water, wax, equipments, and queen.

Variables \ Varroasis		Positive	Negative	p value
Use of additional pollen	Yes	62(89.8%)	7(10.2%)	P=0.00011*
	No	10(91%)	1(9%)	
Purchase pollen from other apiculture centers	Yes	65(91.5%)	6(8.5%)	P=0.2201
	No	7(77.7)	2(22.3%)	
Purchase wax from other apiculture centers	Yes	58(89.2%)	7(10.8%)	P=1.000
	No	14(93.3%)	1(6.7%)	
Purchase second hand equipments from other apiculture centers	Yes	24(92.3%)	2(7.7%)	P=1.000
	No	48(88.9%)	6(11.1%)	
Purchase queen from other apiculture centers	Yes	32(84.2%)	6(15.8%)	P=0.1414
	No	40(95.2%)	2(4.8%)	
Source of water	River	63(88.7%)	8(11.3%)	P=0.0008*
	Non river	9(100%)	0(0%)	

***P<0.05 is significant**

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RAPID SPACIAL AND TEMPORAL OUTBREAK INVESTIGATIONS USING CELL PHONE TECHNOLOGY: A PILOT STUDY

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SUMMARY

The cellular telephone has become an affordable and appropriate means of communication for all levels of society in South Africa, including those with little formal education. Recently, in 2009, constraints to rapid mobilisation in the case of an outbreak of Foot and Mouth Disease were demonstrated, when State Veterinary staff could not reach or communicate with affected livestock farmers because of service delivery protests in the area. Cellular telephone technology has been used elsewhere for monitoring and surveillance of disease outbreaks, as well as for recording field data and transmitting it to a distant server for analysis. This technology, however, requires a well trained technician and equipment that would be too costly for a small scale farming enterprise. The suggested model uses the advantages of appointing a key person (community animal health worker) at each dip tank or village in a Veterinary district. This person is directly linked to the cell phone of the Animal Health Technician and serves as a conduit; both for information required by the State to monitor and control diseases and also information to help the farmers in the area cope with an outbreak. It can also be used to set up appointments or notify communities of information days. Linking this to geographical positioning means that the State Veterinarian or AHT could go directly to the farm concerned, which is currently difficult because of the lack of road signs and road infrastructure in communal areas. This paper describes a novel and practical way to use text messaging linked to geographical positioning, to overcome the lack of Veterinary resources and access to Veterinarians in rural areas, particularly by emerging farmers.

INTRODUCTION

The use of the cellular phone has become popular both in developing and developed countries. In 2008 there were an estimated 4 billion mobile phone subscribers worldwide. As the mobile phone has become cheaper more people are getting access to mobile phones (Cole-Lewis & Kershaw, 2010). South Africa has also seen a decrease in the interconnection fee since the beginning of 2010 and development of low cost handsets (Blycroft, 2008; SATGN, 2010; Taylor & Francis Group, 2008). The penetration of mobile phones in South Africa, by the end of 2007 had increased to 84%, partly because of the increased availability and affordability of this technology for low income consumers (Blycroft, 2008).

Globally the use of mobile technology is not limited to just interpersonal communication, it is being used for health promotions, disease prevention and education (Cole-Lewis *et al.,* 2010; Saxenian & Parikh 2009); Scott *et al*., 2004; Arminnen, 2007; Ramswaroop *et al*., 2010). One example of that is Project Masikuleke, which uses cell phones to communicate with patients that have HIV and TB and increase the testing rate for these diseases (Poptech, 2010). The use of mobile phones for disease reporting as well as human emergencies has become an important tool in epidemiology and disease surveillance. The China Centre for Disease Control (ChDC) used mobile phones to develop an emergency reporting system for infectious disease surveillance after the earthquake in 2008. In their study it took 2-3 minutes to be able to report a case (Yang et al., 2009).

Lack of telephone infrastructure and the theft of copper cables in South Africa, hampers the delivery of technologically advanced methods of communication through telephone lines (Vecchiatto, 2007). The most affordable alternative is mobile technology. Mobile phone users do not need special training and some of the newer models are very user friendly, even for those with limited education (Blycroft, 2008). In the rural situation the difficulty comes in when the farmer or user is illiterate. However, it has been experienced since 1990 that many elderly communal farmers, who were illiterate and innumerate, learned to read and write in order to use a cell phone as these have so many benefits to communication. These benefits include social contact with other family members and the ability to call a medical practitioner or veterinarian in an emergency. In rural families it has been observed that parents and grandparents request the assistance of scholars to help in reading or sending a text message (short messaging service or sms) as well as making calls.

In South Africa, State Veterinary Service delivery is hampered by long distances, poor road infrastructure, cost of fuel and lack of sufficient staff in certain provinces (SAVC, 2010; Van Dijk, 2009). Farms and rural villages do not have street names that make it easy to identify farms (Dhlamini & Dissel, 2005). In the past farmers had to travel to the State Veterinary office to report diseases and deaths in livestock, or to lead the Veterinary staff to their farms. Since the advent of cell phones, farmers phone the State Veterinary offices and are reluctant to come and fetch the Veterinarian (SV) or Animal Health Technician (AHT). It is very easy to get lost on the winding, interconnected dust roads in the rural areas and it can take a long time to reach the destination. Geographical Positioning Systems (GPS) co-ordinates can be used for locating farms and SVs and AHTs now have these installed in their cars. New advanced cell-phones that include GPS technology have been released, but are still expensive and difficult to operate (Cnet, 2010).

For an animal disease surveillance system to be effective, rapid response and mobilisation is critical, especially for rapidly spreading transboundary diseases, such as Foot and Mouth (FMD) (OIE, 2009). In September 2009 a FMD outbreak was confirmed in Mpumalanga Province. However, SV personnel and farmers were unable to cooperate in limiting the spread as there were service delivery riots with blockage of roads in the same area (JakarandaFM, 2009; Modisane, 2010; SABC, 2009; Roberts

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& Sabirovic, 2009). Mobile technology thus offers the possibility of rapid communication between farmers and State Veterinary Services, which could be used to gather spatial and temporal data for epidemiological monitoring, modelling and control strategies. From the above, it can be seen that cellular phones have become affordable and accessible to rural communities and state veterinary services now have access to GPS technology. This pilot study reports on an experiment to develop logistics for the use of cell phone calls and sms, combined with GPS co-ordinates and mapping, to assist SVs and AHTs with surveillance and routine Veterinary Services in rural communities.

MATERIALS AND METHODS

Rust de Winter lies both in Limpopo and Gauteng Provinces and borders on Mpumalanga. The study area for this experiment, Nokeng tsa Taemane Local Municipality, is in Motsweding District Municipality in Gauteng and is about 1975.94 km^2 Rust de Winter forms a small part of Noketsa tsa Taemane, approximately 102 km^2. It is divided into the following farms; Leukraal, Besuidenhoutskraal, Rooikop, Bulfontein, Melkhoutfontein, Kloppersdam, Boschkloof, Zaagkuilfontein, Enkeldoorns Poort, Loodswaai, Loodswaai, Naauwpoort and Kromdraai. Each farm has between two and six camps (Fig 1).

The study was done during an outreach excursion for Veterinary students in the study area, in February 2010. The students (n=120) were divided into 10 groups of 12 people per group. In each group two students were selected, based on their ability to use text messaging or voice calling: in total 48 students. Twenty four farmers were also purposively selected from the study area, based on the criteria that each farmer should have a cellular phone and be able to use sms or voice calling.

A list of farmers was compiled according to surname and farm name, based on the data given by the Gauteng Department of Veterinary Services[1]. Each farm was visited and the following data collected:

1. the full name and contact details of the owner of the farm,
2. the cellular phone number of the farmer ,

The location of the farm using GPS and census data on the number of each species of animals was obtained from the Gauteng Department of Veterinary Services. The number of animals on the farm was categorised by owner, not farm, due to the fact that four farmers could be on the same farm but use different camps. Farmers were categorised according to farm location (GPS Co-ordinates) and the data was then recorded using Microsoft Excel 2007[2]. The database of farmers' names and locations in Microsoft Excel 2007 was linked to Garmin Mapsource[3]. Farmers were given a cellular phone number they could use to contact the AHT or SV. They could use either text messaging or phone calls to report problems seen on their farms or if they needed information on a particular topic during the exercise.

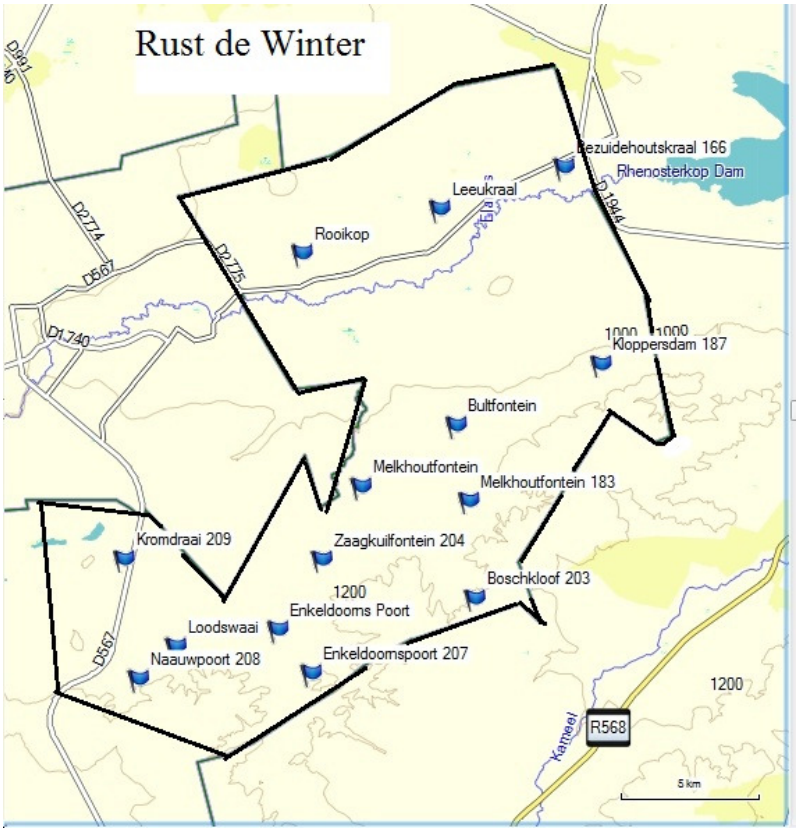

Figure 1: Gauteng area of Rust de Winter showing location of farms in the study

1 Gauteng Department of Agriculture, Veterinary Service, Animal Health Division

2 MicrosoftR Office excel 2007 edition

3 Garmin (Pty) Ltd, Mapsource version 6.15.4

The Faculty of Veterinary Science supplied *Clostridium chauvoei* (Blackquarter) vaccine[1] for cattle and Ivomectin injectable[2] for treating sheep, goats and pigs against internal and external parasites. Dogs were vaccinated against Rabies[3] and Distemper (DHPPI[4]) while cats received Feligen CRP[5] as well as Rabies vaccination. Student groups were supplied with medications and vaccines according to the number of species on each farm and they treated or vaccinated the animals accordingly. Each farmer (N=24) was allocated two Veterinary students whose function was to write a report on the number and type of vaccines used and any clinical syndromes seen per species, using both text messaging and voice calling. They had to include the farmer's cell phone number or surname. Each student (n=48) and farmer(n=24) involved was supplied with sufficient airtime to be able to send reports using text messaging or voice dialling to a central point, where there was an operator with a receiving cell phone linked to a laptop computer.

Data from both text messaging and voice calling was recorded manually on a laptop as it came in, using Microsoft Excel that was linked to Garmin Mapsource version 6.15.4. Thus Geographical Information Systems (GIS) maps were generated immediately as the data was entered into the Excel spreadsheet. Linking a smart cell phone (the make used in the experiment was Samsung i900 Omnia[6]) with Windows Mobile 6.5 software to a HP Pavilion[7] laptop meant that text messages could be received or sent directly to any of the cellular phones on the database.

RESULTS

The reports received during the exercise (n=121) are shown in Table 1. Table 2 shows the number of cases of each clinical syndrome seen. Table 3 indicates the number of vaccines per species administered by the Veterinary students.

Table 1: Total number of reports received from both students and farmers

	Text Messages	Voice Calling	Voice Mail
Students	57	13	9
Farmers	7	20	15
Total	64	33	24

Table 2: Number of cases of each syndrome encountered

Diagnosis	Abscess	Lameness	Heartwater	Peri-anal Laceration	Under Weight	Mange	Ingrown horns	Total
Number of cases	26	3	1	15	12	1	10	68

Table 3: Total number of animals vaccinated

Species	Cattle (Vaccinated Blackquarter)	Sheep treated with Ivomectin	Goats treated with Ivomectin	Pigs treated with Ivomectin	Dogs (vaccinated DHPPI + Rabies)	Cats (vaccinated Feligen CRP+Rabies)	Total
Number of animals	3140	1142	237	241	40	20	4820

A map indicating the distribution of farm owners and the position of their farms in Rust De Winter is shown in Fig 1. Fig 2 shows the location of the farmers – there could be more than one on each farm shown in Fig 1. Fig 3 is a map indicating the vaccination coverage (Blackquarter given to cattle) over a period of 8 hours. A map showing the distribution of peri-anal abscesses seen in livestock, probably due to horn injuries and tick bites, is shown in Fig 4.

1 Onderstepoort Biological Products Ltd, Black Quarter vaccine

2 Pfizer SA Ltd., Dectomax (R)

3 Intervet Ltd, Nobivac Rabies vaccine

4 Intervet Ltd, Nobivac DHPPI vaccine

5 Virbac RSA Pty (Ltd) Feligen CRP

6 Samsung Electronic Co. Ltd, Samsung SGH I900 Omnia

7 Hewlett Packard (R) Development Company, SA Ltd.

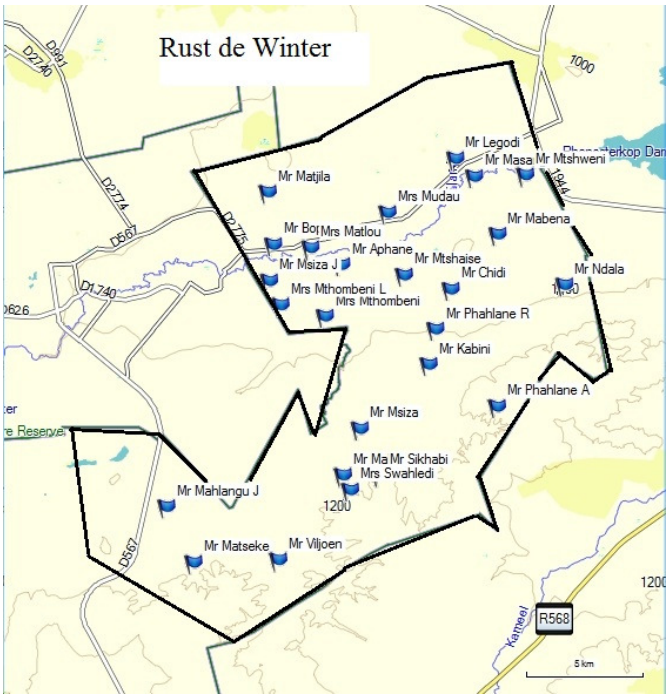

Figure 2: The location of the farmers

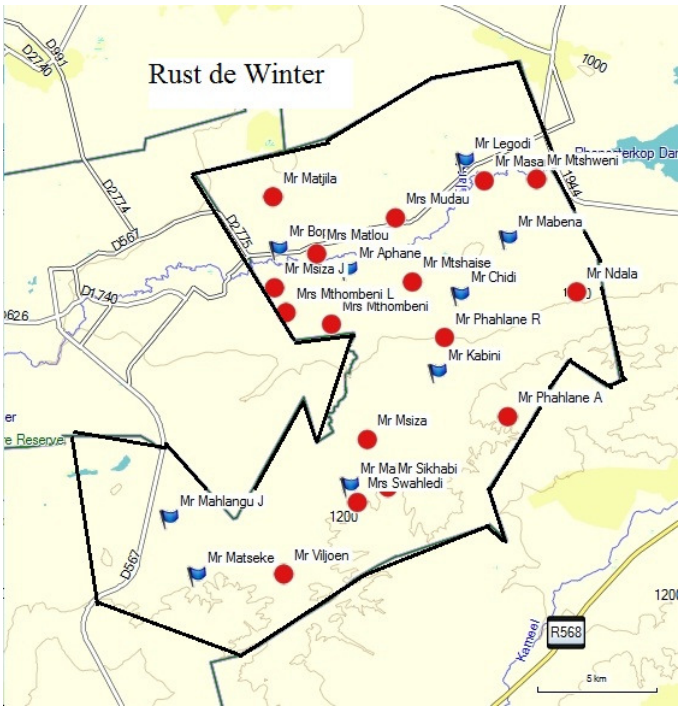

Figure 3: Distribution of cattle vaccinated against Blackquarter (those farms where cattle were not yet vaccinated are shown as flags, while those already vaccinated are shown as red dots). Those farms where cows were not vaccinated during the first 8 hours were vaccinated on a subsequent visit.

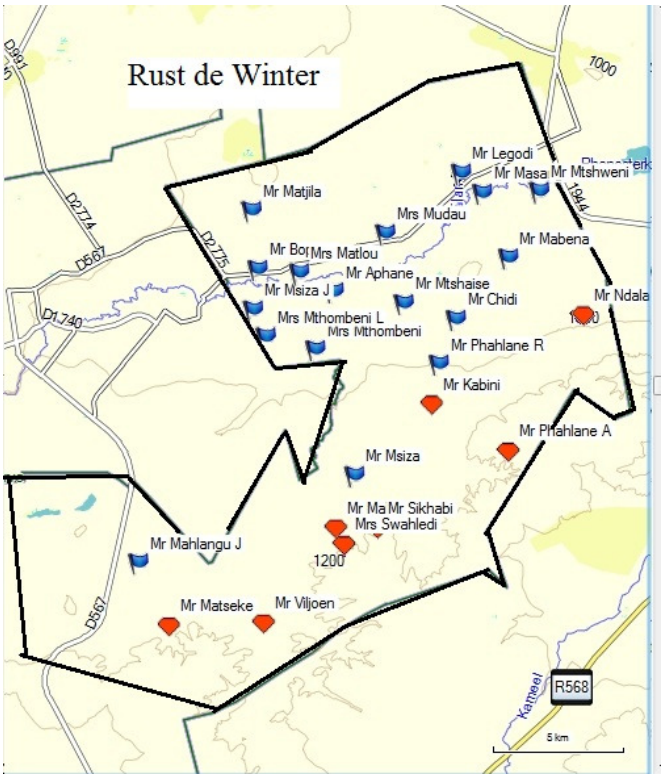

Figure 4: The red triangles indicate farms where cattle had peri-anal abscesses. It was noted that tick burdens were high next to the hills in the same area as the peri-anal abscesses were seen.

DISCUSSION

Access to rural areas currently poses a challenge to both successful disease surveillance and monitoring. For effective monitoring and control after a disease outbreak or disaster, you need a very efficient response system. This must be in place before the outbreak or disaster. Using the participation of community members and modern technology means that communication links with State Veterinary personnel can be maintained during an emergency situation. The GPS readings of the farms are very important as most of the farms in the study area did not have name-boards, nor were there street names or signboards that could direct Veterinary personnel to the location of the farm. In South Africa, as mentioned earlier, infrastructure is lacking and this makes access to rural farmers very difficult. Having the cell phone number of each and every farmer and also a central reporting point for disease could be of great importance. In the case of an outbreak the State Veterinary Services can send batch text messaging to the farmers to alert them and they can also report back to the central reporting point, on the disease or cases as they occur.

In this study students were paired with farmers. Most of these farmers were more familiar with voice calling than text messaging and this is well emphasized by the results we received. Only seven out of 42 reports received from farmers were from text messages as they said that a sms takes a long time to type. They had the ability to use sms but preferred using voice calling. In comparison, 57 sms reports were received from students, who were very quick at typing on cell phones.

One of the problems encountered during the study was lack of signal for mobile phones. It was found that when farmers or students could not report the diseases using voice calling because they could not reach the central point, they resorted to using a sms. In cases like that, sending a text message to the central point was the only effective method of communication, as it could be picked up as soon as the cell phone was moved to an area with better reception. Text messaging was the preferred form of contact from the central station as it could also be picked up as soon as students moved to an area with better cell phone reception. Voice calling was not possible almost half of the time because of the geographical position of the student or the farmer and poor strength of the cell phone signal.

As the cases were reported they were put into GIS software and the maps where drawn as illustrated in the following figures. Fig 2 shows the extent of vaccine coverage of cattle – this could be overlaid with census data to show where there is insufficient coverage in the case of an outbreak. The ability to rapidly draw maps allows for spatial visualisation of the problem, vaccination coverage or temporal spread of an infectious disease. This is well illustrated in Fig 3, which shows the spatial distribution of peri-anal abscesses. Although a few were due to horn injuries occurring in the cattle crush, the majority were more likely due to the bites of long mouthed ticks such as *Amblyomma* and *Hyalomma* spp. Most cases occurred in areas close to the mountains and on farms in close proximity to each other. It was observed that cattle in these areas had a high tick burden. Such information can be used to target tick control strategies and perhaps hold a farmer's day on tick control in the area to decrease the incidence of peri-

anal abscesses. The incidence can then be monitored over period of time as strategic methods are put in place to control the problem. If the number of cases decreases over time it can be seen that the strategy and extension messages are successful – as the farmer will be monitoring his own cattle he becomes involved in the process of tick control and that "ownership" motivates further towards success.

CONCLUSION

Cellular technology is becoming one of the key elements in disease surveillance in Africa, specifically South Africa, and this can be of great importance. There is a need for a surveillance system which is labour and cost-effective. A participatory approach to surveillance and monitoring is supported and farmers can contribute by taking responsibility for reporting cases and ensuring control of diseases of their own animals. Outbreaks or emergencies can be mapped to give early warning and rapid updating of temporal and spatial aspects of a disaster or disease outbreak. Nearby farmers can be alerted and this give farmer to be able to prepare themselves.

Rapid communication using this technology can also be successful in cases where there is a fire, flood or an outbreak of a rapidly spreading disease like Foot and Mouth (FMD). In the case of civil unrest, communication can be maintained even if it is difficult to reach the affected area for controlling disease. This technology, for instance, would have been very useful in assisting with the notification of farmers and putting control measures in place during the outbreak of FMD in Mpumalanga in 2009. It is participatory and empowers both farmers and Veterinary personnel.

Finally, this technology was easy to implement without the assistance of IT experts. Students, Veterinarians, AHTs and farmers could all manage to work with the level of technology needed for effective implementation.

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A USER FRIENDLY DISEASE DATABASE FOR STATE VETERINARY OFFICES

J.L. Kotzé[1]

SUMMARY

State Veterinary (SV) offices obtain large amounts of data regarding (especially) controlled diseases. The data consists mainly of diagnostic results and figures on vaccination and other control measures. This data is an invaluable resource for retrospective research on disease epidemiology, especially where the response of disease occurrence is measured against the control measures applied. In the SV context, this research in turn helps to evaluate and possibly redesign current control strategies. Unfortunately, the raw data within SV offices is often not available on easily searchable databases. Using Visual Basic, a user friendly platform was programmed as an interface between a Microsoft Access veterinary database and users with limited computer literacy. Besides allowing the user to run queries on stored data, the program also has buttons to automatically compile specific sections of the office's monthly reports. This is proving to be a strong incentive for SV offices to make use of the program. The program is currently being implemented in the Ehlanzeni district, Mpumalanga.

INTRODUCTION

The need to organise data through the use of a database is well recognised.[3] At the state-vet office level, this need is particularly important in order to make sense of large quantities of available data. A well functioning state veterinary office has the potential to avert animal health, human health and economic disasters where the efficiency of such an office often hinges on making informed decisions based on reliable data. At the policy making level, strategies are determined by what the data from state veterinary offices reveal. Therefore data integrity and availability is of the utmost importance. However, data availability is often the primary constraint to epidemiological based decision making.[2] Many state veterinarians are unable to provide data on the prevalence of relatively common diseases in their areas and are unable to statistically evaluate the effectiveness of their control efforts despite spending immense sums of money on surveillance and control efforts. One reason for this problem is perhaps that state veterinarians are bogged down by among other things administrative work and that their work has been reduced to the compilation of different reports. This has led to the demise of the evaluation and interpretation of disease prevalence and their time-spatial tendencies. To combat these concerns a user friendly database was designed to facilitate easy capturing and easy retrieval of data. The aim at the start of the design process was to save time at all stages of data management (see figure 1), enable users with no experience in database use to easily store and retrieve data and to stimulate an interest in disease epidemiology through automated special reports.

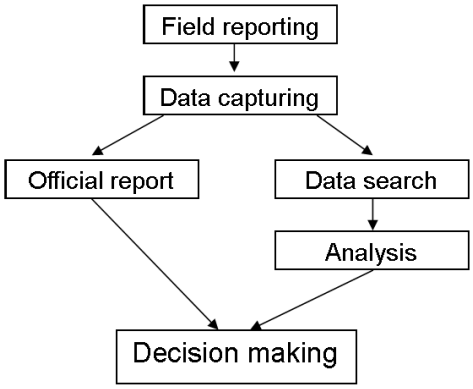

Figure 1: A schematic representation of the flow of data. The disease database is primarily involved with data capturing, official reports and data searches; however it directly affects data analysis and decision making.

MATERIALS AND METHODS

The backbone of the database was created using Microsoft Access 2007. Separate tables were created to contain testing/surveillance/laboratory data, vaccination data, extension/education data and permits/documents data. The entries in these tables combine with numerous relational tables containing information about species, diseases, farms, owners etc. Several shortfalls in the database had to be resolved using Visual Basic for Applications 2007 (Microsoft). This entailed more than 10 000 lines of code and several trial runs of different versions. Table 1 lists some of the problems encountered and how they were solved. Some of those problems and solutions are discussed in more detail here.

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Table 1: Some of the solutions to some of the problems encountered during the design and initial testing phases of the database.

Problem	Solution
Different names for the same diseases/species/places and difficulty in working with codes.	Drop-down lists with common name, standard name and codes next to one another. The user has the ability to register or change the 'common name'.
Making duplicate entries by mistake.	If a reference is entered that is been used before an automated warning flag is displayed with summarised data of previous entry.
Reliability of data capturer.	Captured data can be validated by doing a search on data using the user name and/or date captured as parameters for the search.
Database security.	Different access levels and capabilities for different users. Users log in with a password. The administrator decides what level of access is allowed for every user.
Difficulty in reporting results of tests that were run months before. (For example TB culture results.)	Ability to change the month that data will be reported in.
Tediousness of typing full names.	Predictive text completes the entry for the user.
Monotony of capturing large sets of similar data.	Buttons that duplicate previous data entered.
Users not familiar with query language.	Simplified queries with prompting buttons.
Confusing old back-ups of the database with the latest version.	A user history report is provided that shows recent activity as well as the sums of different kinds of entries.
Further editing of reported data.	Data is reported in Excel sheet ready for editing. Changes made are not reflected in database.
Unwillingness of offices to make use of database.	Automated official reports save days worth of time.
The danger of entering nonsensical data.	Various flags have been built in to make the user aware of unexpected entries. For example entries where the number of positively diagnosed animals does not agree with the number found positive to a specific test.

See figure 2 for a typical screen shot when a disease result is added to the database. The sampling date automatically generates a suggested report month that can be changed if the user wants to delay the reporting of the result (often due to pending secondary tests.) When the user proceeds to enter references, these are immediately checked and the user alerted with a summary of a previous entry with the same reference. This limits the duplication of results in the database. The next parameter that is selected is the farm, which can be selected or typed (with predictive text assistance). Once the farm is selected the owner list is automatically updated to only include owners registered to the farm/locality. Other automatically updated fields are 'Responsible person' and 'Ward'. These fields can be changed if the default settings do not apply; however having them automatically updated greatly increases the speed with which entries can be added. It is important to mention that any disease, farm, owner etc that is not registered can easily be registered while adding a new entry by simply following the prompts that appear when an unfamiliar name is used.

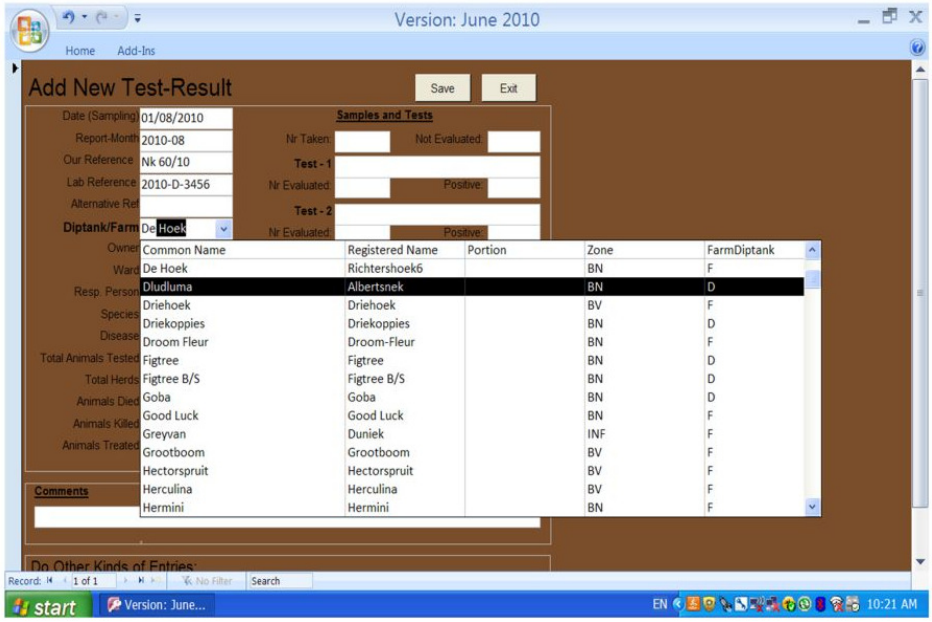
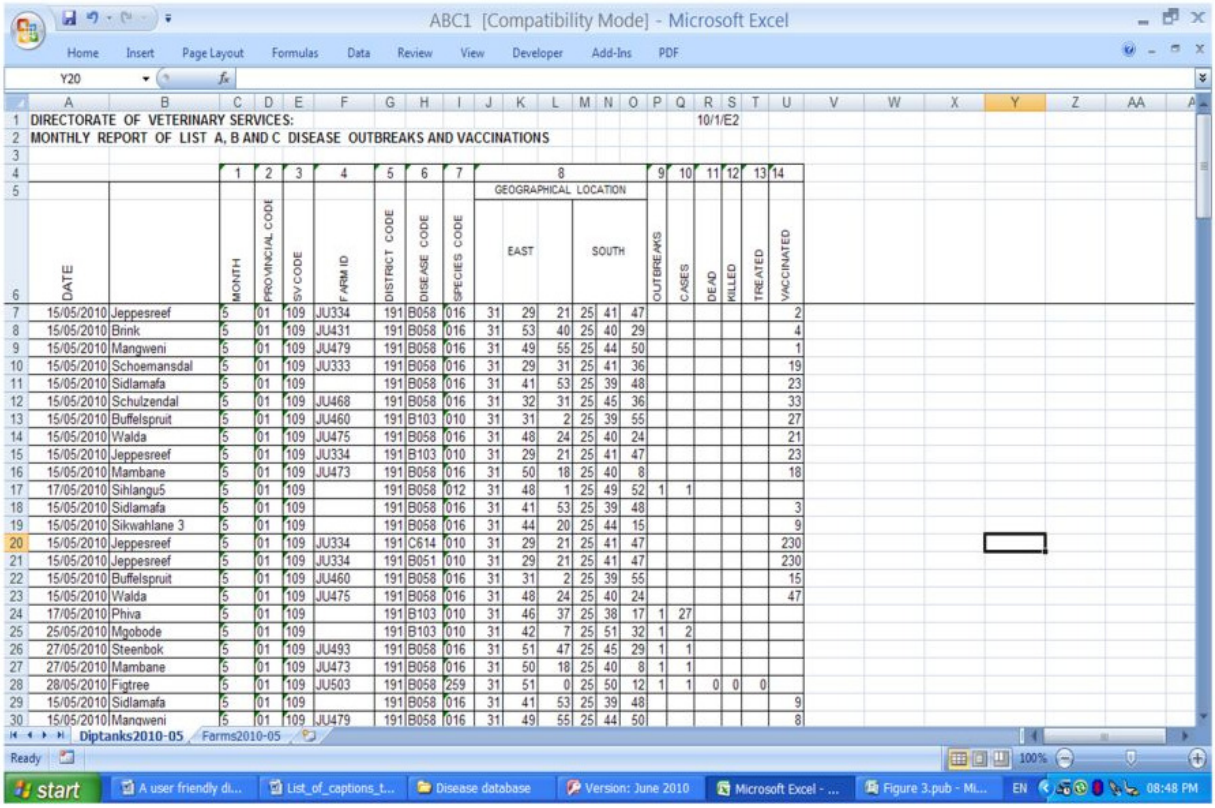


Figure 2: A typical screen when a disease result is added. Note the drop down list selected for farms where the different columns convey various details about the farm. Once a farm is selected the owner list will be automatically updated for that farm.

It was decided for the sake of allowing users the freedom to edit the results of the database, that all reports should be exported into Excel files. These Excel files can then be manipulated without affecting the original data in the database.

RESULTS

The database has thus far been implemented in two offices in the Ehlanzeni district of Mpumalanga where the transition from established methods is proving to be difficult. However the massive amount of time that is saved through automated reports has helped to overcome any reluctance to implement change. The compilation of a set of monthly reports that used to take two people three or more days to complete now takes approximately 8 man hours only. (See figure 3).



ABC1 [Compatibility Mode] - Microsoft Excel

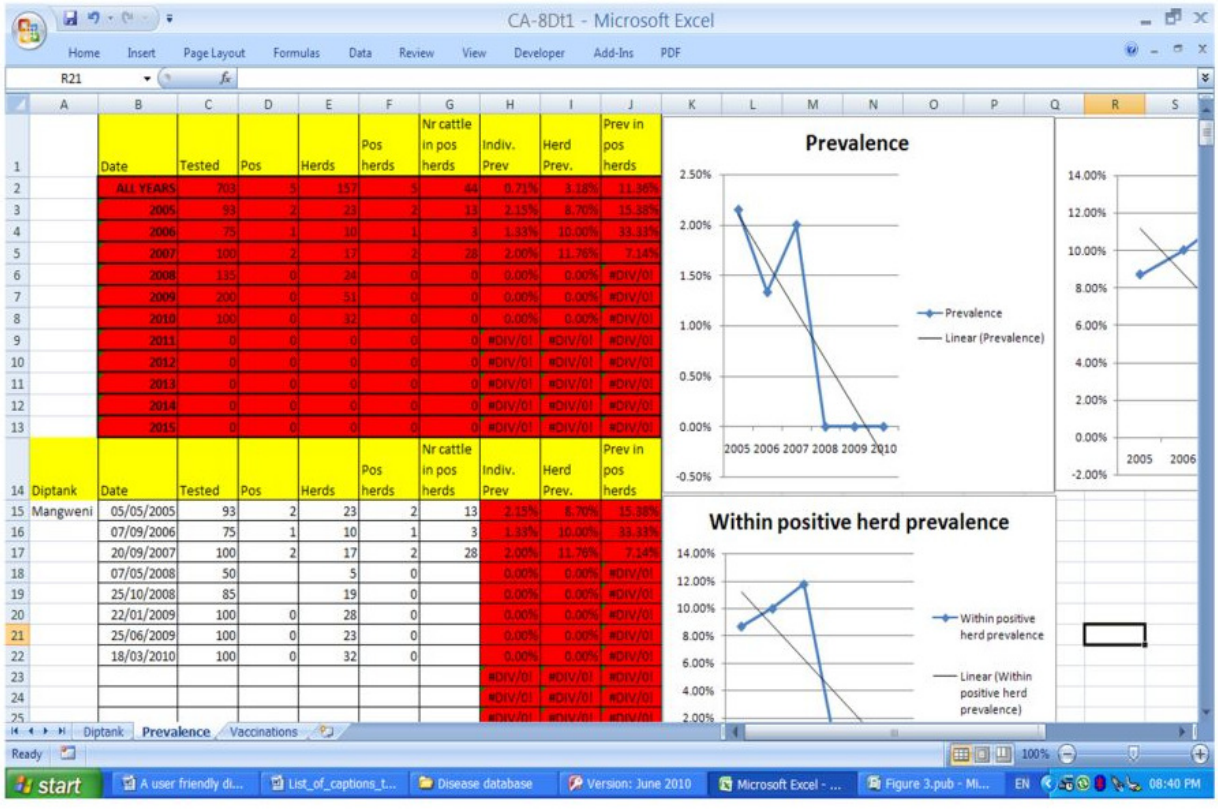
DIRECTORATE OF VETERINARY SERVICES: 10/1/E2

MONTHLY REPORT OF LIST A, B AND C DISEASE OUTBREAKS AND VACCINATIONS

DATE		MONTH	PROVINCIAL CODE	SV CODE	FARM ID	DISTRICT CODE	DISEASE CODE	SPECIES CODE	GEOGRAPHICAL LOCATION EAST		SOUTH		OUTBREAKS	CASES	DEAD	KILLED	TREATED	VACCINATED
		1	2	3	4	5	6	7	8				9	10	11	12	13	14
15/05/2010	Jeppesreef	5	01	109	JU334	191	B058	016	31	29	21	25	41	47						2
15/05/2010	Brink	5	01	109	JU431	191	B058	016	31	53	40	25	40	29						4
15/05/2010	Mangweni	5	01	109	JU479	191	B058	016	31	49	55	25	44	50						1
15/05/2010	Schoemansdal	5	01	109	JU333	191	B058	016	31	29	31	25	41	36						19
15/05/2010	Sidlamafa	5	01	109		191	B058	016	31	41	53	25	39	48						23
15/05/2010	Schulzendal	5	01	109	JU468	191	B058	016	31	32	31	25	45	36						33
15/05/2010	Buffelspruit	5	01	109	JU460	191	B103	010	31	31	2	25	39	55						27
15/05/2010	Walda	5	01	109	JU475	191	B058	016	31	48	24	25	40	24						21
15/05/2010	Jeppesreef	5	01	109	JU334	191	B103	010	31	29	21	25	41	47						23
15/05/2010	Mambane	5	01	109	JU473	191	B058	016	31	50	18	25	40	8						18
17/05/2010	Sihlangu5	5	01	109		191	B058	012	31	48	1	25	49	52	1	1				
15/05/2010	Sidlamafa	5	01	109		191	B058	016	31	41	53	25	39	48						3
15/05/2010	Sikwahlane 3	5	01	109		191	B058	016	31	44	20	25	44	15						9
15/05/2010	Jeppesreef	5	01	109	JU334	191	C614	010	31	29	21	25	41	47						230
15/05/2010	Jeppesreef	5	01	109	JU334	191	B051	010	31	29	21	25	41	47						230
15/05/2010	Buffelspruit	5	01	109	JU460	191	B058	016	31	31	2	25	39	55						15
15/05/2010	Walda	5	01	109	JU475	191	B058	016	31	48	24	25	40	24						47
17/05/2010	Phiva	5	01	109		191	B103	010	31	46	37	25	38	17	1	27				
25/05/2010	Mgobode	5	01	109		191	B103	010	31	42	7	25	51	32	1	2				
27/05/2010	Steenbok	5	01	109	JU493	191	B058	016	31	51	47	25	45	29	1	1				
27/05/2010	Mambane	5	01	109	JU473	191	B058	016	31	50	18	25	40	8	1	1				
28/05/2010	Figtree	5	01	109	JU503	191	B058	259	31	51	0	25	50	12	1	1	0	0	0	
15/05/2010	Sidlamafa	5	01	109		191	B058	016	31	41	53	25	39	48						9
15/05/2010	Mangweni	5	01	109	JU479	191	B058	016	31	49	55	25	44	50						8

Figure 3: A section of a detailed OIE (List ABC) report that would normally have taken hours of work is completed within seconds.

The biggest job that remains is the capturing of data and the validation thereof. Furthermore, the completion of CA8 forms (a compilation of historical surveillance data used to report back on Brucellosis tests) used to take several minutes or more depending on the availability of data, but these forms now take no more than a couple of seconds each to compile. In addition, the CA8 reports have been modified to contain more data, thereby providing a better understanding of the disease process on individual farms. Graphs generated automatically from the database have given greater insight into the effect of S19 and Rb51 vaccination on the prevalence of *Brucella abortus* infections in cattle. (See figure 4). Before the database implementation this amount of data processing would have taken weeks, it now takes a couple of seconds!



CA-8Dt1 - Microsoft Excel

	Date	Tested	Pos	Herds	Pos herds	Nr cattle in pos herds	Indiv. Prev	Herd Prev.	Prev in pos herds
ALL YEARS		703	5	157	5	44	0.71%	3.18%	11.36%
2005		93	2	23	2	13	2.15%	8.70%	15.38%
2006		75	1	10	1	3	1.33%	10.00%	33.33%
2007		100	2	17	2	28	2.00%	11.76%	7.14%
2008		135	0	24	0	0	0.00%	0.00%	#DIV/0!
2009		200	0	51	0	0	0.00%	0.00%	#DIV/0!
2010		100	0	32	0	0	0.00%	0.00%	#DIV/0!
2011		0	0	0	0	0	#DIV/0!	#DIV/0!	#DIV/0!
2012		0	0	0	0	0	#DIV/0!	#DIV/0!	#DIV/0!
2013		0	0	0	0	0	#DIV/0!	#DIV/0!	#DIV/0!
2014		0	0	0	0	0	#DIV/0!	#DIV/0!	#DIV/0!
2015		0	0	0	0	0	#DIV/0!	#DIV/0!	#DIV/0!

Diptank	Date	Tested	Pos	Herds	Pos herds	Nr cattle in pos herds	Indiv. Prev	Herd Prev.	Prev in pos herds
Mangweni	05/05/2005	93	2	23	2	13	2.15%	8.70%	15.38%
	07/09/2006	75	1	10	1	3	1.33%	10.00%	33.33%
	20/09/2007	100	2	17	2	28	2.00%	11.76%	7.14%
	07/05/2008	50		5	0		0.00%	0.00%	#DIV/0!
	25/10/2008	85		19	0		0.00%	0.00%	#DIV/0!
	22/01/2009	100	0	28	0		0.00%	0.00%	#DIV/0!
	25/06/2009	100	0	23	0		0.00%	0.00%	#DIV/0!
	18/03/2010	100	0	32	0		0.00%	0.00%	#DIV/0!

Figure 4: A typical (unedited) screen shot of the Excel file CA-8 report for a village called Mangweni. Note that there are various tabs within the file containing their respective information on vaccinations, prevalence and a finalized CA-8 report. This report is generated within seconds.

DISCUSSION

Essentially the database is a success; it is however pending the successful implementation in multiple offices. Numerous benefits have already been gained from its implementation. Some of the major benefits include drastically reducing the time spent to prepare reports, comparative work reports for animal health technicians, indications on the effectiveness of brucellosis control programs and the relative need for rabies vaccinations in different localities. Regardless of these benefits, the potential for improvement and expansion is limitless. I suggest that the next step should be to incorporate animal census data. This would enable statistical calculations of measures of certainty with regards to disease presence or prevalence. These results in turn would enable risks to be calculated for the spread of disease. In the Foot-and-mouth and Corridor disease control zones, it is not necessary to wait for official animal census data, instead the data from cattle registers from dipping and inspection records can be used as dynamic census data. This can be used to alert state veterinarians of population tendencies that might indicate the presence of insidious diseases like tuberculosis or contagious bovine pleuropneumonia. In the early nineties a major outbreak of zoonotic *Escherichia coli* O157 in humans from Swaziland, was preceded with three months by drastically increased mortality rates in cattle.[1] Retrospectively these mortalities are reckoned to have been related to the outbreak in humans. However, if an efficient computerised data management tool had been available to alert state veterinarians, investigations could have been made that might have averted a serious zoonotic epidemic. The ideal would be that disease databases within the state veterinary services combine with information from various corners of the private sector to create a national disease database with valuable information not only with regards to controlled diseases but all animal health aspects. The first step to move towards that ideal is creating stand alone databases for state veterinary offices. Lastly it is hoped that this disease database will cultivate an interest in epidemiology among state veterinarians. It is also hoped that the database might play a role in making the position as state veterinarian a more appealing option for young veterinarians. This in turn might ultimately help to fill the alarmingly high veterinarian vacancy rate of 37% (2008 data) within the state.[4]

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WESTERN CAPE ANIMAL DISEASE NOTIFICATION SYSTEM – A WEB BASED SPATIAL SURVEILLANCE SYSTEM

J.D. Grewar[1*] & F.C. Basson[1]

INTRODUCTION

Web-based disease surveillance is undertaken using various methods throughout the world, with different systems set up for different requirements. These systems make use of data from scientific forums like ProMed mail and WHO (World Health Organisation) as well as public and media data sources such as Google News and Reuters. There are systems like HealthMAP[2] and EPISpider[1] that use manual data input and/or automation which filters different news sources for any reports of animal disease. Most systems rely on visual reporting in the form of maps. The Western Cape Animal Disease Notification System (WCADNS) is a web-based system of inputting disease outbreak and prevention data that has been tailor made for the State veterinary services in the Western Cape. This manual user input system makes use of a combination of map inputs and outputs with an associated form to capture required data. The fact that the system is web-based allows for continued development with concurrent user access, this being an improvement on standalone spreadsheet and database systems. Current users of the system are predominantly animal health technicians (AHT's) as well as state veterinarians, in future we envisage access to private veterinarians who would be able to log and retrieve data. Preliminary results in the Western Cape are extremely promising with an increase of user activity in the region of 400% compared to the previous stand-alone systems.

MATERIALS AND METHODS

SYSTEM COMPONENTS

The WCADNS is an online interface that integrates various web and database technologies. The web page is hosted on an Apache web server while the database is maintained on a secure server within the Provincial Department of Agriculture network. The Apache web server, commonly referred to as Apache, is a popular and reliable open-source HTTP (Hypertext Transfer Protocol) server[10]. Apache provides the functionality to host dynamic and static web pages and supports server-side programming languages such as PHP (Hypertext Preprocessor)[3].

The system requires interaction from the client as well as processing on the server side. Client-side interaction is handled with JavaScript, while PHP is used to perform the necessary server-side processing and to manipulate the database. JavaScript is the scripting language that is used to access web page objects on the client side[5]. The Google Maps Application Programming Interface (API) also requires JavaScript to construct and modify the Google Maps interface. JavaScript is also used for error checking of the data that the user submits and controlling the information displayed in the dropdown selection lists. PHP is a scripting language that is used for web development to create dynamic web pages[6]. PHP can be embedded in HTML (HyperText Markup Language), but requires interpretation by a web server with a PHP processing module. In this case the Apache web server hosts the PHP processing module. PHP is used in the web application to handle user sessions, construct SQL (Structured Query Language) queries for reading and writing to the database and dynamically construct web pages required to enter and retrieve data. As mentioned, PHP executes from within the server environment.

The visual mapping component utilises the Google Maps API to capture and display the spatial distribution of events. Google Maps is a familiar web mapping service, which can be embedded in a web page[4]. The Google Maps API is required to manipulate the Google Maps interface in terms of loading the map, changing the view type, adding and removing map data, setting zoom scales and changing the view extent. The API is completely controlled by JavaScript.

All events are captured and stored in a Microsoft® Access database (© Microsoft Corporation). The database was originally designed as a stand-alone application to capture and retrieve event information. A MySQL database could be a better and open source alternative to the Microsoft® Access database. An Open Database Connectivity (ODBC) instance is used to access the database for reading and writing. ODBC allows the execution of standard SQL queries on the database. All database interaction is handled through queries created with PHP.

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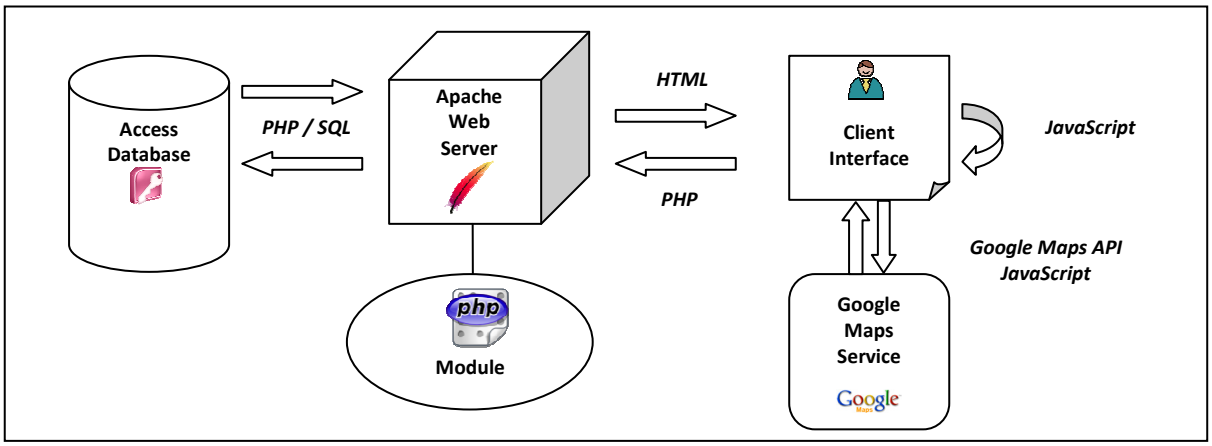

Figure 1: Diagram illustrating the interactions and scripting required between components of the WCADNS

RESULTS

CLIENT INTERACTION

DATA INPUT

Upon access, the user is prompted for a username and password to continue to the main data capturing page. The username does not only serve as a security feature, but is also used to maintain user specific information during the session. The user is then be taken to the main capturing page where the data capturing form and Google Maps interface is displayed (see Figure). Event information is entered, with data required being spatial variables in either DMS (degrees, minutes, seconds) or decimal degree format, date (month) of event, disease name and species name. The disease and species lists are populated dependently of each other. If a disease is species specific (an example being African horse sickness) the underlying code will only allow input of the specific species affected by the disease (in this example only "*Equine*" will appear on the *Species* dropdown list). Events are then classified into *Outbreaks*, *Follow up* (of outbreaks) and *Routine vaccination* events. A comments input is also available for data entry. All data inputted fulfil the requirements of the South African Department of Agriculture, Forestry and Fisheries' provincial monthly data reporting, and disease and species lists were populated based on these requirements. These data are required for reporting to the OIE (World Organisation for Animal Health) regarding OIE listed disease information.

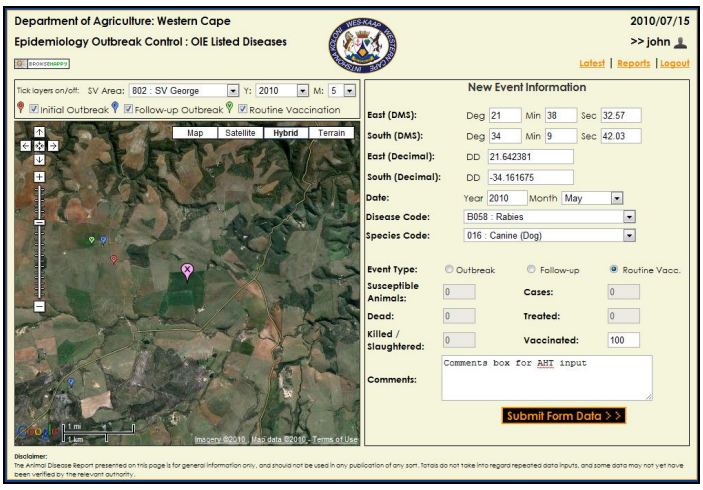

Figure 2: General screen capture view of the WCADNS

New event information is internally checked for input errors when submitted from the main page. The new information is then sent to the Apache server, where it will be inserted into the Microsoft® Access database through a PHP/SQL query. Data is immediately available to view (see Figure), with selected aspects of each point available by clicking on the icon on the map where the event is located. Icons are colour coded with red indicating *outbreaks*, blue indicating *follow ups* and green indicating *routine vaccinations*. The initial screen also has some user filtering function where different areas, different months and/or different events can be selected. Users are automatically shown their particular state vet area when they log on with data shown on the map for the current month.

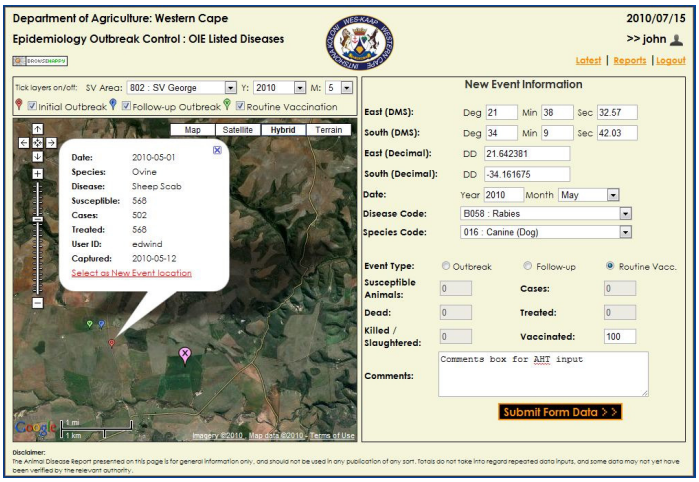

Figure 3: Data submitted is available for viewing immediately, with limited data available when clicking on the icon on the map where an event of interest occurred.

ADMINISTRATOR FUNCTION

The index page has links to other sites, one of which aids management in monitoring use of the website. A query is run which shows events classified into *outbreaks*, *follow up* and *routine vaccinations* per month of entry and State Veterinarian area. This assists in monitoring usage and stimulates timely input of data by users, who previously entered these data at the end of every month.

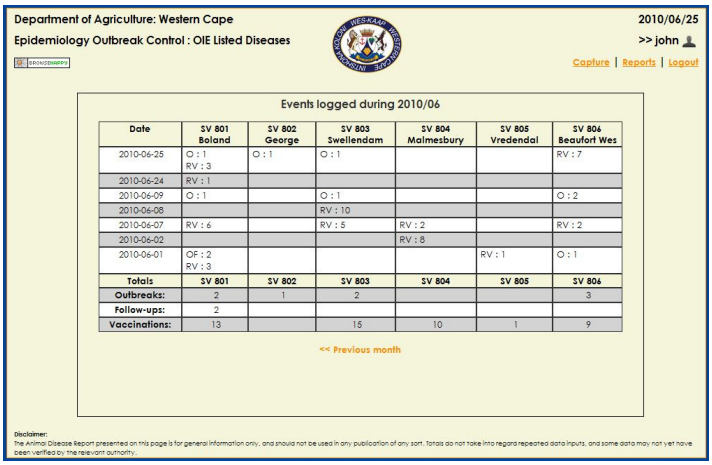

Figure 4: Usage and area of usage query run to assist management in ensuring timely data entry

DATA REPORTING

A user input query option has been added so that users can access specific data which is then graphically depicted on a static map which can then be downloaded and printed or used in presentations.

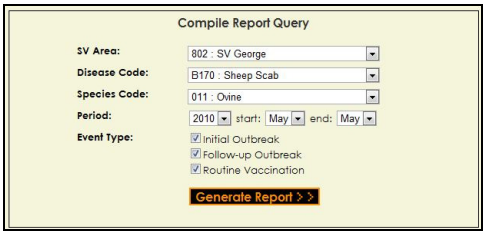

Figure 5: User defined query for disease and species specific information

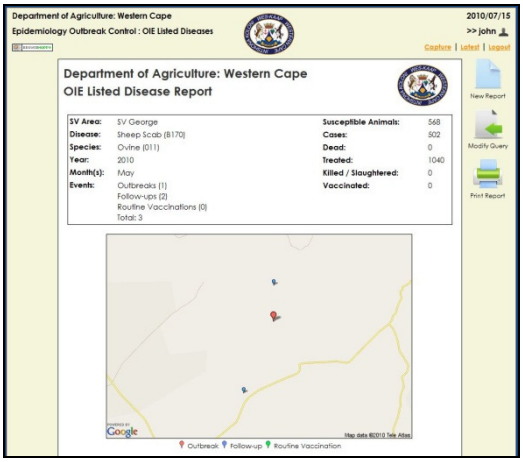

Figure 6: Output of user defined query for disease and species specific information. Note the options to print/modify or to re-draw the query.

The performance results of the system are limited, with only 3 completed months of the fully functioning website where all users have logged on and logged events. Preliminary results do however show a dramatic increase in the number of events logged on the system. This can be seen on the graph below where the final 3 months of logging are on the web based system. Over time these types of results will be more enlightening, and it is expected that the graph will flatten out at the new higher level of utilisation. It is important to note that Rift Valley Fever (RVF) events accounted for 48, 112 and 36 events in March, April and May 2010 in the Western Cape Province respectively.

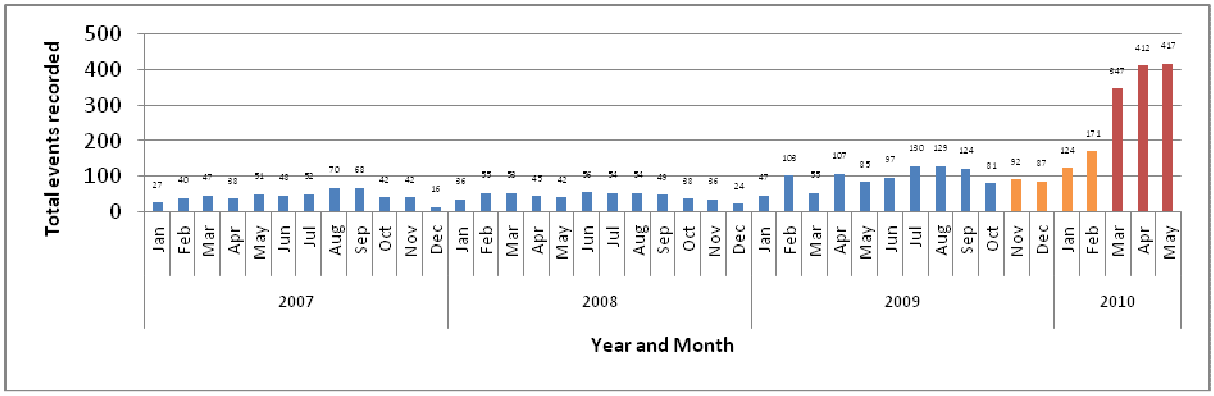

Figure 7: Count per month of entries of disease outbreak and vaccination data recorded by the Western Cape Department of Agriculture, Veterinary Services: Animal Health. Blue columns (prior to Nov 2009) represent data capture via Microsoft® Excel spreadsheets, Orange columns (Nov 2009 – Feb 2010) represents data input via local databases while Red columns (March 2010 – May 2010) represents data input using the WCADNS.

DISCUSSION

The feedback from users has been very positive in terms of ease of use of the system. However, with implementation of a new system like this training was performed, and with training comes a new impetus to log disease data. It is difficult to say whether this drive has pushed up the figures or whether it is primarily a function of the user friendliness and access of the web system. It is likely a function of both and in time when reporting on the web is the standard process an evaluation of the monthly figures can be re-assessed. The other factor to consider with the large increase in cases is the recent RVF outbreak which increased disease and vaccination events, especially in the Beaufort West area. Although these were a large quantity of events, they still do not account for the sharp rise in events after implementation of the system. It is also difficult to say whether, in the absence of the web based system, RVF events would have been logged to this extent.

Data integrity is maintained on the system as far as possible using queries within the web page accessed by the users. An example of this is the filtered dropdown lists for *disease* and *species* selection. Also if the event is a *routine vaccination,* fields which are not applicable are 'greyed out' and no input is possible there. However, as with any user-input system, we expect some level of human error on WCADNS.

Most web based disease surveillance systems currently available make use of multiple source input, and the focus of these systems is identifying outbreaks of disease early enough to prevent its potential spread, and the method of achieving this is via multiple news services and alert systems[9]. The WCADNS differs from this type of system in that it currently has user access control and

data is only entered by registered users, these currently consisting of AHT's and state veterinarians. Based on how the system progresses a private veterinary login may be used in future. We envisage a system that has registered private veterinarians who have a simplified disease input form, where data entry is easy and minimal training will be required. The accuracy of disease surveillance data must always be weighed up against the timeliness of data acquisition. For surveillance data to be useful it must be early on enough in an outbreak where control methods can make an impact on the spread of disease. Accuracy has been considered by systems such as HealthMap where it was shown that the HealthMap automated classifier had an accuracy of 84% with accuracy for ProMED alerts being 91% compared to Google News reports at 81%. The current WCADNS estimated accuracy is high, with the majority of reports being sourced by state officials who performed the disease investigation or vaccination. As soon as outside sources of data are introduced into the system the accuracy thereof is bound to diminish, as much of these sources will not be able to be verified. However, with the decreasing accuracy an increased impression of disease and disease vaccination will be attained for the Province, which is to the advantage of the general surveillance of disease in the area.

Having a system which is situated on a major server has disadvantages and advantages. As can be seen in Figure the inputs after migrating to the web based system were significantly more than when a spreadsheet or local database system was used ($p<0.05$). The advantage of the web system here is that all officials have access to the server and the internet provided by the Departmental IT solution. The major disadvantage is that any problem which occurs with the main server affects all users on that server. Our system has had downtime as a result of server related issues. It is also important to note that should the system need to be hosted on a server that is linked to a company or Governmental department, knowledge is required of open source systems that are described above. The major advantage of this geographic information system is that all the software required to create it is open source. The only exception is the Microsoft® Access database on our system, but there are database programs which are available as open source software which will also suffice.

Data privacy and confidentiality is an important consideration when mapping disease information[7]. With the current system which is user limited this consideration is not yet necessary as all users have access to confidential information without needing to try to acquire it from the website. However, if the system is made open to private veterinarians and other public institutions, some type of data protection will need to be considered. Options like data aggregation into specific human defined boundaries like local municipalities or spatial skewing are two methods which will have to be considered[8].

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KNOWLEDGE MANAGEMENT AS A TOOL CONTRIBUTING TOWARDS AN EFFECTIVE ANIMAL - HUMAN ECOSYSTEM

(Filling the gap between the status of Good Health and Disaster -a perspective from the Eastern Cape Province)

S.H. Kroll-Lwanga-Iga[1] & I. Lwanga-Iga[1]

SUMMARY

The ever changing global environment with its significant increase of the risk of transboundary and emerging disease outbreaks created the urgent need to holistically address the challenge of disease outbreak prevention, preparedness and readiness to respond appropriately, effectively and timeously within the existing limitations of scarce resources and the country's unique challenges created by its socio-economic structure to support a healthy balance in the Animal – Man ecosystem, to ensure food security and prevent economic hardship.

The current increases in both important economic and zoonotic disease outbreaks in South Africa and the Eastern Cape Province in particular, affect the poorest of the poor in all communities in the country most significantly as regards to their livelihoods, wellbeing and continued socio-economic development.

South Africa in general and Eastern Cape in particular are characterized by vast communal, rural areas with subsistence and emerging farmers intertwined with highly commercialized farming establishments, the closeness of people to animals in general, the size of areas in need of State Veterinary Support Services, the accessibility challenges of villages, distances between farms as well as the scarcity of available resources in terms of skilled people, infrastructure and finances.

This paper proposes an innovative Knowledge Management system with two main components, namely the system in terms of an appropriate, customized, electronic, integrated, interactive, spatially enabled, real-time, mobile, modular database system and the institutional support environment from the State Veterinary Services perspective. It looks at the current requirements of quality data production, access to information, practical aspects of optimizing available, appropriate technologies, and maximizing scarce resources on one hand and the challenge to promote active participation of all stakeholders in the sector on the other. The paper is intended to initiate and promote serious discussions on the role of appropriate Knowledge Management systems in addressing South African challenges in veterinary service delivery and respectively as a contribution towards an effective Animal – Man Eco-System. (FAO, OIE, WHO, 2010)

KEYWORDS

Knowledge Management Systems, Data quality, Public participation, Holistic, collaboration

INTRODUCTION

During the last two decades the young post-apartheid government of South Africa and its State Veterinary services have experienced the serious consequences of global environmental changes, bringing along with them increased risk of introduction of transboundary and emerging diseases as well as changes in patterns of disease outbreaks and transmission.
The Eastern Cape Province, one of nine provinces in South Africa, had to prevent the spread of Foot and Mouth Disease across the borders from neighboring Kwazulu Natal in 2000, dealt successfully with an outbreak of Avian Influenza in ostriches in July 2004, endured the challenges of eradication interventions fighting Classical Swine Fever in pigs since August 2005 and suffered the professional dangers of zoonotic diseases like Rift Valley Fever in 2010.
These recent major events did not stop occurrences of other diseases like Rabies, Bovine Brucellosis, African Horse Sickness and Sheep Scab amongst others.
Considering the composition of the farming sector ranging from subsistence to emerging and commercialized systems and the topography of the Eastern Cape Province with vast communal rural areas, barely accessible in wet weather conditions, with large numbers of livestock and pets in need of veterinary services, the closeness of the rural population with animals in general and the poor available infrastructure in the areas of the former Homelands, it is therefore not surprising that the State Veterinary services have been stretched beyond their limits to "kill burning fires" while at the same time attempting to continue rendering basic services and implementing the annual Primary Animal Health Care campaigns.
Such massive interventions required temporary movement of staff and resources to hotspot areas to assist with the crises while other areas suffered a "form of neglect" due to man-power and logistical shortfalls.
While disease control legislation is in place in South Africa, it could be argued whether the prescripts for implementation of such, are appropriate and logistically possible in some of the terrain of the Province or the diverse country as a whole.

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In other words, these unexpected, non-scheduled disease occurrences interrupted the natural flow of service delivery, thereby allowing for diseases previously satisfactorily managed, flaring up undetected, besides the extra demands on the staff component.

This is where the notion of Veterinary Knowledge Management as a tool contributing to a more effective and efficient service delivery comes in. But, what is Knowledge Management, and what role should it play in the context of State Veterinary Services in the Eastern Cape and South Africa as a whole? In order to understand the different concepts of and discussions around knowledge management it is important to define the meaning and the differences between Data, Information and Knowledge.

DEFINITIONS

Data, information and knowledge are not the same and each has distinctive characteristics, but is not seen in a linear hierarchical structured relationship for the purpose of this paper, as data can become information in certain situational context (Alavi, 2000).

DATA is raw facts out of context and can appear as alphanumeric characters, image, audio or video. In order to make data valuable it has to be accurate, complete, economical to produce, flexible in terms of utilization purposes, reliable, relevant, simple, timely, verifiable, accessible and secure from unauthorized use. Data by itself has little meaning.

INFORMATION can be defined as processed data, which means it has been changed through various processes to make it useful in the context of a specific problem situation.

Information can be classified, analysed, reflected upon and processed thereby becoming knowledge (Blankert et al., 1997). Information answers to the "what- when- who and where" questions and entails understanding of relationships.

There is no consensus on what constitutes **KNOWLEDGE**, but some of the following definitions attempt to express the essence of the meaning:

According to Alavi (2000) knowledge is justified belief that increases an entity's potential for effective action. This means that "…knowledge is information of which a person, organization or other entity is aware..." and can be created by experience, learning, perception or through association and reasoning. (Wikipedia, 2006 and 2010)

In the context of the current economy, knowledge is, according to Drucker (1992), the "…only important resource today…" and is "… key to the power shift…", in other words the only meaningful economic resource.

Knowledge is all about people and can only be volunteered and not extracted by means of force or otherwise. It relates to strategy, practices, methods or approaches and answers to the "how" question.

This is supported by Duffy (2000) who says that knowledge is a product of as well as an input into processes and work. The value of knowledge is added by people in form of context, experience and interpretation, thereby transforming data and information into knowledge.

The relationship between data, information, knowledge and intelligence/wisdom could be described as follows: Analysed raw facts (Data) become information, which becomes knowledge if synthesized. Knowledge which undergoes a contextual phase becomes intelligence or wisdom.

Schreiber et al. (2001) caution, that information and knowledge can only add value, if there are practical and positive applications with the aim of enhancing service delivery in general and the quality of service delivery specifically for all members of society.

There are three types of knowledge, tacit, implicit and explicit. Conversion between the types occurs according to Nonaka's SECI model of knowledge creation (Gourley, 2003) in four distinct modes.

TACIT KNOWLEDGE not accessible to consciousness. People know more than they can say (Knowledge Harvesting Inc., 2001 Gourley, 2003). It answers to the "Know How?" question.

IMPLICIT KNOWLEDGE like tacit knowledge resides inside the brains of people, but it is known although difficult to verbalise and express. Knowledge Management aims among others to assist through processes to access this knowledge that people can talk about, but have not yet expressed, in other words to make implicit knowledge explicit (Knowledge Harvesting Inc., 2001; Gourley,2003).

Explicit knowledge has been recorded or codified as either text, audio, video or graphic and is thereby easier accessible and distributable (Knowledge Harvesting Inc., 2001; Gourley, 2003). It has to be noted that only a small fraction of knowledge will be spoken or written down. Explicit knowledge answers the "Know what?" question.

Explicit knowledge always has underlying tacit and implicit knowledge and a clear distinction or differentiation of the types of knowledge is consequently not scientifically possible.

WHAT THEN CONSTITUTES KNOWLEDGE MANAGEMENT (KM)?

The term Knowledge Management is a new buzzword since the mid 1980's and is extremely difficult to define due to the fact that there are many different approaches and perspectives to the management of knowledge which is only represented as physical virtual entity of knowledge in its explicit form. KM is commonly used to describe the process of locating, organizing, transferring and using information (Duffy, 2000:10), but will for the purpose of this paper be defined as "…a framework and tool set for improving the organizations knowledge infrastructure, aimed at getting the right knowledge to the right people in the right form at the right time."(Schreiber et al., 2001:72). These authors describe activities making up the knowledge process as elements of a "coherent whole" (holistic view).

According to the brint.com website, KM is the management focusing on change, uncertainty and complexity (theory of complexity) through synergy of people, processes and technology, thereby re-focusing on so called "human-sense-making" processes (systems thinking theory) which are influencing decisions, choices and performance. Consequently such synergy between the human creativity and innovative capacity with the information-technological capacity of data and information processing is seen as a possible solution to the need for constant organizational adaptation to the ever changing, complex and competitive global environment; therefore enabling translation of data and information into meaningful decisions and actions for a sustained performance.
This notion is supported by Duffy (2000) who states that knowledge management is about managing the quality of knowledge content through a combination of good technology with right cultural and people changes, carefully guided by a full time knowledge manager in order to leverage the organisation's knowledge. He goes on to say that knowledge supports the four cornerstones of successful competitive organizations today, which are learning, partnerships, leadership and agility (Senge, 1990).
Wiig (1997) summarises systematic Knowledge Management as multi-dimensional and broad in scope, covering most aspects of activities of organizations and defining success as a function of quality of knowledge content.
According to Campbell et al (1999) the study or discipline of knowledge management "…evolved from the need for companies to manage resources more effectively in a hyper-competitive, global economy." He describes KM as a dynamic process, which is supported by Alavi (2000) who states that "… knowledge management consists of a dynamic and continuous set of processes and practices embedded in individuals, as well as in social and physical structures."
Many models have been developed to understand underlying principles of processes from knowledge creation to valuable sharing, distribution and utilization of knowledge. Most of the models identify the core function of KM as the process of "seed (sow)-grow-harvest" (Prudlatz, 2000).

The main KM objectives are therefore according to Rowley (1999):

1. The creation of knowledge repositories with added value through "…categorization and pruning…",

2. Improvement of the access to knowledge through increased connectivity, transfer processes and expert networks,

3. Enhancement of the knowledge environment by implementing processes promoting creation - transfer and use of knowledge with emphasis on collaboration and promotion of individual creativity through organizational structures and cultures,

4. Management of knowledge as an asset which has to be monitored and measured for performance through the impact of the use of knowledge. The Balanced Scorecard system, developed by Kaplan and Norton (1992) with four dimensions addressing the customer, internal processes, innovation and learning as well as financial aspects is seen by Rowley (1999) as a possible tool used to measure indirectly what is not really measurable directly.

Appropriate knowledge management can lead to bottom-line savings, improve operational efficiency, create higher productivity, support organizational learning and innovation in products and services and where applicable increase revenue and decrease "time to market" aspects of delivery (Clarke, 2001).

The question then arises, why do we need knowledge management, especially in the public sector, today? Due to the current trend of globalization and its related escalating speed of change, increasing competition to unprecedented levels, it has become clear that competitive advantage can no longer be found in access to natural resources or improvements to operational efficiency alone.
The unintended consequence of universal ease to information access in a world connected through the internet and other networks have made the knowledge in people, embedded in experience, the most important asset for achieving competitive advantage. This appreciation of intellectual assets, the tacit knowledge situated in people, is at the core of the so called "knowledge society".
In order to find answers to these problems, knowledge intensive skills or cognitive skills, which can not be replicated by silicon chips, are necessary to add value to the organization. This knowledge-centric view of organizations makes knowledge, mostly tacit and implicit knowledge, the main building blocks for successful organizations.

HOW THEN DOES KNOWLEDGE MANAGEMENT FIT IN THE CONTEXT OF THE PUBLIC SECTOR, ESPECIALLY IN THE NEW SOUTH AFRICA?

The post apartheid democratically elected Government of South Africa is still in a process of major, rapid transformation from an exclusive to an inclusive Public Service addressing the needs of all citizens and aiming at wiping out the imbalances of the past by improving the quality of life for all. Challenges range from addressing the enormous service delivery backlogs of the past to instilling a culture of Batho Pele and tolerance in a society with cultural diversity, many different languages, beliefs and value systems.
All of these have to be achieved with limited financial and human capacity resources, fulfilling the social responsibility as mandated by the Constitution of the Republic of South Africa (1996) in a democratic manner and at an accelerated speed.
Consequently, it becomes clear that knowledge and maximal utilisation of such a scarce resource are critically important for strategic planning and improved decision making processes thereby avoiding unnecessary duplication and repetition of previous costly mistakes. Knowledge Management is concerned with strategy and tactics to manage such intellectual capital, creating value from this socially constructed intangible asset (McAdams and McCreedy, 1999)

MANDATE / LEGAL FRAMEWORK OF THE STATE VETERINARY SERVICES

The Legal framework consists amongst others of the following:

THE ANIMAL DISEASES ACT, ACT 35 OF 1984 provides for measures to promote animal health and to control animal diseases, establish animal health schemes and provide for various matters related thereto.

THE MEAT SAFETY ACT, ACT 40 OF 2000 provides for measures to promote meat safety and safety of animal products, establishment and maintenance of national abattoir standards, establishment of meat safety schemes and provisions for various matters related thereto

THE ANIMAL IDENTIFICATIONS ACT, ACT 6 OF 2002 provides for systematical identification of livestock and ownership thereby enabling tracing and contributing to fighting stock theft

THE SPATIAL DATA INFRASTRUCTURE ACT NO. 54 OF 2003.
Objectives of the Act are to establish SASDI (South African Spatial Data Infrastructure) and a CSI (Committee for Spatial Information) in order to determine standards and prescriptions with regard to the facilitation of the sharing of spatial information, to provide for the capture and publishing of metadata as well as the establishment of an electronic metadata catalogue and consequently to avoid costly duplication of capture of spatial information. The Act explains the multi-facetted role and responsibilities of a data custodian in Sections 11, 12, 14, 15, 16 and 18. These include adherence to prescripts and standards, availability of metadata, aspects of accountability, copyright and integrity, updating frequency, data security, unauthorized access etc. as well as the data user or vendor in section 17 regarding notifications about identified deficiencies

THE INTERGOVERNMENTAL RELATIONS FRAMEWORK ACT, 2005 (ACT NO. 13 OF 2005) defines intergovernmental relations as any relationships that arise between different governments or between organs of state from different governments in the conduct of their affairs. Objective and purpose of the Act is "…to provide within the principle of co-operative government set out in Chapter 3 of the Constitution a framework for the national government, provincial governments and local governments, and all organs of state within those governments, to facilitate co-ordination in the implementation of policy and legislation According to Mentzel and Fick (1996:101) intergovernmental relations can be defined as "…a mechanism for multi and bi-lateral, formal and informal, multi-sectoral and sectoral, legislative, executive and administrative interaction entailing joint decision-making, consultation, co-ordination, implementation and advice between spheres of government at vertical as well as horizontal levels and touching on every governmental activity….". Intergovernmental relations touch on institutional, political and financial interaction processes between the spheres

THE STATISTICS ACT, ACT 6 OF 1999
The Eastern Cape Province signed a memorandum of understanding with the Statistics SA (NSS, National Statistics System) on the 23 November 2007 to become a partner in the drive for quality data production. There are 8+1 dimensions of SASQAF (South African Statistical Quality Assessment Framework) which are the following: Pre-Requisites of quality data production (which include Identified Legal Mandate and need, Project Plan and/or Programme with clearly identified responsibility officers, Standards & Policies framework, Adherence to Privacy and Confidentiality , Regulations on implementation of Data sharing and Coordination, Monitoring of Effective and Efficient use of data, Regular quality checks), Relevance of data, Accuracy of data, Timeliness, Accessibility, Interpretability, Coherence, Methodological soundness and Integrity

SOME CHALLENGES IDENTIFIED WITH THE CURRENT SYSTEM

Past experiences, especially in recent serious disease outbreak situations demonstrated the weaknesses of the existing environment, of which some are listed here:

1. Lack of baseline data (most importantly detailed spatially enabled Census data)
2. Late reporting/ notifications (especially in cases where EDW and RR should get involved to implement contingency plans)
3. Incorrect GIS references/ calibrations
4. Insufficient system for follow up reports on reported suspect or confirmed outbreaks
5. Data collectors often un-traceable (very important in the international trade, Exports)
6. Unreliable data/ many discrepancies between original source and reported data (during various compilation processes)
7. Unsatisfactory/ incomplete or lacking metadata
8. Lack of linkages between case (field) and Laboratory reports
9. Lack of uniform naming conventions, especially for place names
10. Lack of uniform reporting format
11. Lack of available realistic, relevant and timeous Stats to meet the needs of Government and other users
12. Lack of quality of data as described in the SASQAF standards thereby not complying with the Statistics Act, Act 6 of 1999
13. Lack of shared , common standards to allow for comparison of data
14. Lack of capacity to produce quality data
15. Many isolated uncoordinated producers of data (fragmentation)
16. Non compliance with SDI (Spatial Data Infrastructure) Act, Act 54 of 2003 (especially regarding data custodianship and metadata capturing)

The Public Service is also plagued with high staff turn-over and loss of experienced staff (non-conducive work environment, lack of retention planning, early retirement packages, HIV/AIDS etc.) resulting in a vacuum of expertise, leadership, and constant loss of organizational knowledge. In addition, the vastness of the country and the partial inaccessibility of some geographical areas present major challenges in achieving participatory democracy which requires continuous availability of appropriate information to a variety of target groups and stake holders.

The physical and organizational infrastructures often lack evenly distributed (across the Province) access to relevant information and communication technology in many instances hence most field animal health services are rendered from mobile facilities.

Although innovative skills of personnel are required in principle, the strictly hierarchical structures prevent any real promotion of innovative thought which might lead to inability to introduce new changes or promote remedial actions timeously.

THE WAY FORWARD

The challenge is to develop knowledge management systems which address the need for reliable, timeous statistics and facts as well as the behaviors of personnel (people) in the system and eventually, in the words of Thompson et al. (2004), “increase the organizations ability to support knowing” thereby ”promoting generation of meaning amongst employees.”

It is clear by now that Knowledge Management in the context of State Veterinary Services is not just another techno-centric Information System which runs as software on computers (in the worst scenario implemented from top down) but a complex and dynamic process to allow for collaboration, participation, alignment and integration focusing on organizational support systems and taking people and their behaviors into account (ecological focus on people).

What does that mean in the context of the existing unit in the Eastern Cape Province, ADSU, an Animal Diseases Surveillance Unit, a specialist unit in the State Veterinary Services of the provincial Department of Agriculture and Rural Development, which is offering professional services to proactively prevent and monitor Animal Disease outbreaks and react appropriately and timeously to these occurrences?

The vision of this unit for the State Veterinary services of the Eastern Cape Province comprises of two main components, a technological highly customized support system and an institutional support environment.

Malhotra (2003) indicates that a knowledge society “…shifts emphasis to beliefs, behaviour, and actions that are more directly related to performance outcomes,…” and demonstrates growing interest in “….knowledge economies and societies that can promote holistic, social, cultural, economic development and well being of citizens…”.

A potential solution is seen in the strengthening of the existing Animal Diseases Surveillance Unit (ADSU) and its upgrading to an institutionalized ***Centre for Animal Diseases Information, Analysis, Alert and Response*** (CADIAAR) comprising of a knowledge management coordination and a mobilization component situated at decision making levels in the structure of the Department of Agriculture and Rural Development with the mandate to prepare the stakeholders in the wider animal sector for emerging Animal health threats and disease outbreaks as well as contributing towards local Provincial and National efforts to prepare for and respond to Animal Health emergencies and disease outbreaks.

CADIAAR’s responsibilities should include to professionally guide and advise the State Veterinary Services in the collection, management, analysis and dissemination of information that is critical to Veterinary Services programs, utilizing Knowledge

Management Principles and creating a platform for knowledge creation and data warehousing through facilitation of interaction between role-players at all levels of the services, departmental, interdepartmental, national and international.
CADIAAR could accomplish this mission by developing collection, storage, management and analysis systems for use in highly customized disease management programs like the proposed ADIMS (Animal Diseases Information Management System with integrated spatial component), providing scientific risk assessments which incorporate epidemiological, geospatial, and agri-economic analyses; and promoting the appropriate utilization of such information and knowledge through customized training programs.
This proposal is also aligned with the need for Departments to start producing quality data according to NSS (National Statistical Services) SASQAF standards and the recent legislation (SDI Act) on spatial data custodianship.
Quality control processes have to be implemented at all levels of the conversion from data to knowledge and must form an integrated part of the system.
Seven principles of cultivating communities of practice (Wenger et al, 2002) which are built on "aliveness" of members have to be followed and comprise of the following:

1. Designing for evolution providing for lifelong learning, promotion of change and dynamic development in general.
2. Creating a platform for open dialogue between inside and outside perspectives because only insiders truly appreciate the issues at the heart of the challenge.
3. Inviting and promoting different levels of participation and connect various stakeholders with the assistance of a coordinator who accepts that different stakeholders have different levels of interest.
4. Developing both public and private community/ stakeholder spaces
5. Focusing on values which is the key to community life
6. Combining familiarity and excitement through dialogue and exposure to unpopular perspectives
7. Creating a rhythm for the involved and interested parties and members adapted to the cultures and needs of the members, thereby keeping the excitement but not overwhelming the participants.

While CADIAAR could be the nucleus of a veterinary module within a bigger institution of the department, it requires positioning that enables it to have a holistic view of the required strategic objectives and other developments influencing the direction and functioning of the Department as a whole. It could be the "missing link" between "peace time" normal Animal Health/ Veterinary Services operations and Disaster Management scenarios, "War Times".
The Knowledge Management component comprises of a Veterinary Informatics Unit facilitating the technical enabling environment (including development of appropriate mobile solutions) and administrative management of the software system, the Veterinary Archiving Services responsible for recording explicit organizational information and knowledge and last but important the Veterinary Early Disease Warning Unit providing epidemiological analysis of surveillance and disease data, developing disease models and issuing bulletins and alerts.
The second component holds the Veterinary Rapid Response Unit which is mandated to ensure adequate disease outbreak preparedness (including the 72 hours initial response, emergency store management, facilitation of simulation exercises, SOP and contingency plans development and maintenance and training amongst others) and the Veterinary Outreach Programme Unit, which in peace times, facilitates uniformity of extension messages, visibility of the Veterinary Services, and produces and collates new audio-visual extension materials but carries out Social Facilitation Coordination in outbreak situations ("War Times").
A separate Quality control unit should be mandated to monitor adherence of required standards and procedures in the whole Directorate.
CADIAAR nodes should be distributed strategically to work with each of the six administrative areas (District Municipality Level of the Department) and create the platforms for active participation and interactive consultative processes. These nodes should also convert to Disease control nodes in disease outbreak situations and provide quality monitoring abilities. These are seen to be very important in the promotion of people centered approaches of the system.

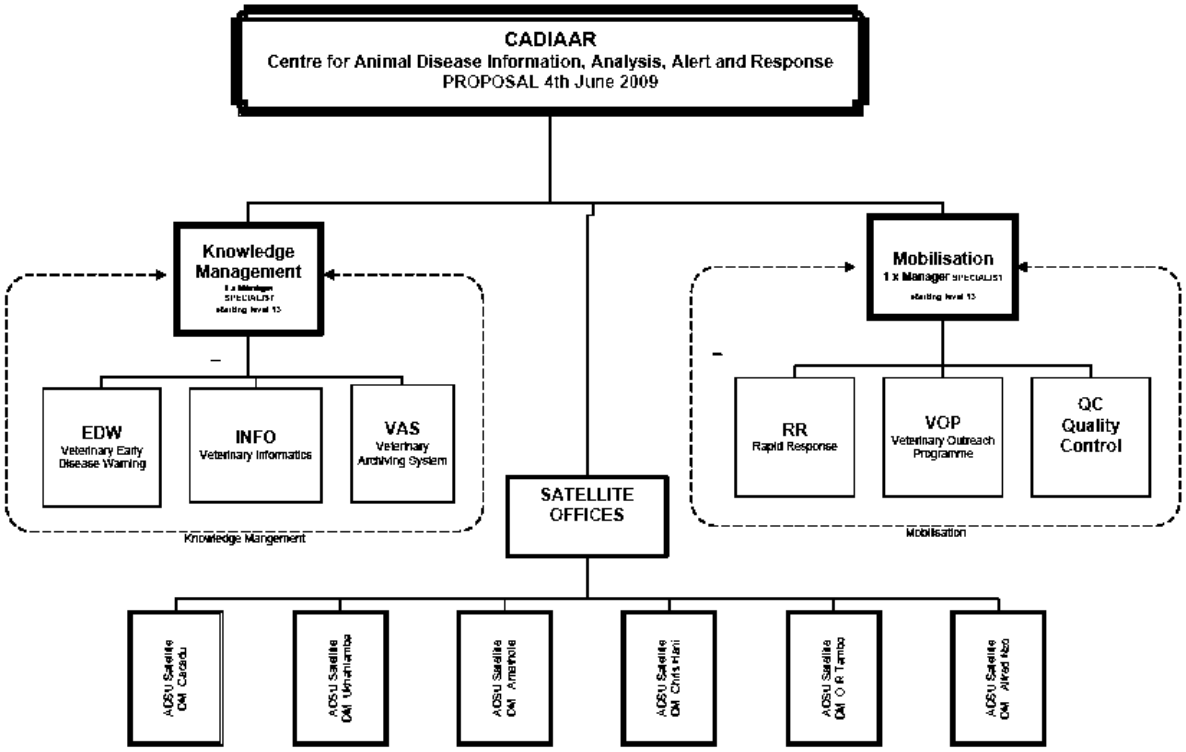

Figure 1: By ADSU-Knowledge Management

Special features of the envisaged ADIMS (Animal Diseases Information Management System) are the utilization of a variety of technological tools with the following objectives:

1. To achieve near real time reporting through mobile technology,
2. To provide an interactive platform for knowledge transfer and consultation,
3. To link SQL database capabilities with spatially enabled GIS systems in an integrated database system,
4. To ensure inclusivity, meaning that data-collectors become a responsible part of system with managers at verification levels and scarce skilled specialists in centrally situated but accessible locations
5. To Interface a variety of mobile devices suitable for the various levels of participation in the system (multiple devices ranging from Digital pens, cellphones, scanners, digital cameras, PDAs, GPSs to laptops)
6. To capture and process spatially referenced data with extensive metadata and allow for information and knowledge creation
7. To facilitate advanced data analysis & enhanced and faster reporting capabilities
8. To cater for internal and external portals to enhance information distribution and exchange beyond Veterinary Services employees.
9. To provide customized, user-friendly and appropriate interfaces for role-players at different levels without the necessity of intensified training exercises or the requirement of sophisticated, technical skills.

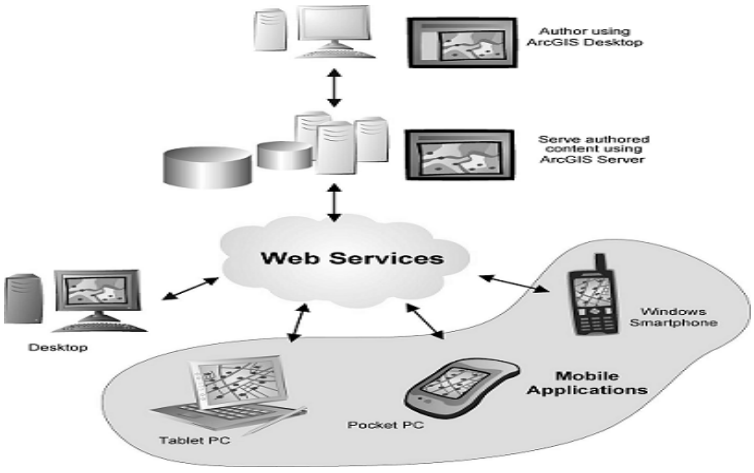

Figure 2: from the www.ESRI.com ArcGIS website

The GIS portion of the system acts as a tool that allows users to create interactive queries (user created searches) through visualisation, analyze the spatial information, edit data, maps, and present the results of all these operations.
A key function in any animal health information system is the role of the spatially referenced data, information and knowledge in improving the quality of disease reporting to decision makers, thereby enhancing administrative management, control programme planning, priority setting and international reporting requirements as well as enabling effective rapid response (to minimise the effects of disease outbreaks), allow for quick access to all the relevant essential information and improve the holistic knowledge through exploration of spatial relationships
Such reports aim to convey a realistic picture of the current disease situation to decision makers and are based on verifiable, quality data using maps, to complement textual or tabular reports and thereby conveying a clear message effectively in a fraction of the time conventional reports would take.
The assumption is that better quality of communication of the animal health information should lead to a better understanding of the current, acute situation and consequently more efficient, informed decisions.
The principle of the CADIAAR functionality utilizes a combination (80% codified to 20% tacit) of the following two main knowledge strategies or models as described by Alavi (2000):

1. Codification Strategy (repository) centering around technology and managing mainly explicit knowledge, the documenting of explicit knowledge in a people to document approach, and the
2. Personalisation Strategy (network model) focusing on information and knowledge sharing through person to person contacts and thereby managing tacit knowledge.

While databases and analytical tools to interrogate such repositories, combined with increased accessibility and connectivity of role-players, especially veterinary staff members, increase the availability of existing, captured knowledge and information for better decision making processes and consultation, the people centered tacit knowledge management aspects would enhance professional knowledge through expert directories and interventions to facilitate active communication and sharing of experience and knowledge.

OBSTACLES TO INTRODUCING KNOWLEDGE MANAGEMENT

There are many potential obstacles or barriers which could prevent the successful implementation of knowledge management in an organization.
Inhibitors , according to Alavi (2000) can range from a competitive environment (suppressing collaboration), lack of individual motivation (in terms of contributing to/ sharing of and using of knowledge), the “silo” syndrome (with lack of contact, relationships and common perspectives among people), to a whole spectrum of cultural frictions, behavior, distrust and intolerance preventing information and knowledge sharing. Lack of business purpose, poor planning, inadequate resources, lack of accountability and customization, conflict between organizational processes and KM, lack of appropriate technology and skills in KM techniques as well as lack of commitment by senior management can also contribute to the failure of KM implementation.
The staffing “crisis” in the public sector also contributes to the situation that time becomes a constraint to KM as well as a culture of unwillingness to share, especially in environments without organizational incentives and rewards for sharing.
Finally there is lack of funding which could be caused by the fact that it is difficult to place a direct financial benefit on successful KM interventions unless impacts are measured or negative consequences of lack of KM are costed.

ENVISAGED BENEFITS AND AREAS WHERE KNOWLEDGE MANAGEMENT COULD ADD VALUE

Knowledge is power and has therefore political implications. KM is also expensive and requires about 7-10% of the revenue base of an organization as a financial resource.
Hierarchical structures suppress the knowledge development cycle and slow down adaptation to changing environments. KM requires multi-disciplinary skilled knowledge workers and a full time "egoless" knowledge manager to oversee and guide the continuous, never ending process of organizational learning.
KM is a theory and a practice and is all about people and learning, therefore it requires a culture which recognizes tacit knowledge and encourages knowledge sharing and interaction between people. It is crucial to introduce knowledge management concepts in such a manner that all staff members buy into the idea and do not feel threatened by "surrendering" their knowledge which makes them valuable to the organization (Levinson, 2006).
The challenge of KM lies in the constant updating, amending and deleting of knowledge management content as well as in the filtering of information aimed at preventing information overloads or unintended erasing of the context necessary for valuable utilization of information and creation of new knowledge.
Integration of Knowledge Management principles and strategies could therefore add value to the institutions mandate by acting as a "think tank" in professional veterinary matters and establishment of best practices, standards and norms within the directorate. Well managed repositories would enhance the advisory capacity of the unit for use by decision makers.
Good Knowledge Management practices could enhance multi-dimensional connectivity to a multitude of role-players and experts across directorates and departments as well as to society at large. The unit could act as a facilitator in the promotion of creativity and innovation, as well as increased communication and information sharing and thereby assist with the establishment of an enabling environment to produce new knowledge, thus promoting participation and "aliveness" of stakeholders. Geographical separation and distances could be overcome through utilization of appropriate customized technology to allow exchange of information and knowledge thereby improving communication.
Knowledge management is essential for effective and efficient early diseases warning and rapid response systems aimed at preventing major epidemiological disasters. Rapid response and general appropriate preparedness is based on maximal utilization of professional knowledge within the global context, allowing for appropriate and speedy adaptation of generic contingency plans to local specific problem situations.(FAO-OIE-WHO, 2010; DEFRA, 2002)
Last but not least, organizational learning processes could be encouraged and valuable individual knowledge converted into organizational knowledge before staff members leave the service for one reason or another (exit debriefing). Such organizational knowledge can then be better utilized in induction programmes for young and new members freshly joining the service.
Reporting could become more meaningful and would include aspects of quality of service delivery through customer satisfaction surveys and built in feedback loops.
According to Malhorta (2003) and the FAO-OIE-WHO (2010) intangible assets like knowledge depend on communication, collaboration and coordination. These essential features would be promoted by CADIAAR to create a platform for social (behaviour), human (intellectual) and sociological capital to work in harmony towards the common goal of improving the learning capacity of the organization.
Four key features make up the so called "knowledge foundation" of successful "high road" modern organizations (Neef, 1999):

1. The knowledge based strategy which includes the development of the organizations agility and addresses the cost effectiveness of labour and location
2. A knowledge sharing culture which seeks to establish a KM framework aligned to the organizational strategic purpose at all levels, utilizing tools like knowledge mapping, communities of practice networks and re-designing reporting lines around people networks.
3. A technical support infrastructure in order to support generic knowledge processes through most suitable methods
4. Business research and analysis to ensure that information is placed in context thereby making it meaningful and relevant as well as by supporting the ".need to know more, with more certainty, more quickly."

This notion is supported by Nissen (2002) who emphasizes that the flow of information and knowledge across time, location and organizations is critical to the organisation's efficiency and performance.
While this proposal is designed for the Eastern Cape Province, the principles could be applied at various levels in different degrees in other provincial and national establishments.

CONCLUSION/ RECOMMENDATIONS

In conclusion, Knowledge Management has the potential to empower organisations in transformation, including the public service, to render better quality, more effective and efficient services to the public in order to better the quality of their lives.
The benefits of properly implemented Knowledge Management Systems (not just information management software systems) outweigh the apparent barriers or inhibitors towards it, especially in complex environments or areas experiencing scarcity of human resource capacity on one hand and massive delivery mandates on the other.
There is certainly room for its implementation in the Department of Agriculture and Rural Development in general in our Province.

A specialized, provincially based institution like CADIAAR utilizing an information management system like ADIMS in the Eastern Cape Province, if successfully implemented could act as a model for expansion countrywide in order to enhance the quality of service delivery of its professional Veterinary Services and escape the so called "Silo" syndrome.
It is important to remember that no single system or approach unless systematical and holistic in nature can achieve the required outcomes unless the "people factor" becomes an integrated part of the system.
This proposal also assumes that a general strategic direction for the State Veterinary Services exists and is expressed in National and Provincial Strategic Plans and Policy documents. There are good and working examples internationally in other countries like Australia (AUSVET and NAHIS), Britain (DEFRA), Germany (TSN), but it should be remembered that importing such systems en-masse would not address the local challenges since no system can exist without willing and contributing participants. The tacit knowledge specific to the South African way of life is within our people and needs to be given a platform to interact and become organizational knowledge.
This paper intended to trigger active and lively discussion on the KM concepts which will hopefully lead to development and implementation of appropriate "local" solutions towards an effective Animal – Man Eco-System.

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