Southern African Society for Veterinary Epidemiology and Preventive Medicine

Proceedings

Second Annual Congress

22 – 23 July 2002

SOUTHERN AFRICAN SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

Proceedings of the second annual congress held at the Exotic Disease Centre, Onderstepoort, Gauteng, South Africa on the 22nd and 23rd July 2002

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The views expressed in these proceedings are not necessarily those of the Editors or the Executive Committee of the Society.

ISBN 0-620-29288-1

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PAPERS

DEMOGRAPHY AND DOG-HUMAN RELATIONSHIPS OF THE DOG POPULATION IN NORTH CENTRAL DIVISION OF NAMIBIA

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SUMMARY

In the North Central Division of Namibia (NCD), rabies is becoming an increasing problem, above all for human health but also for the economic damage it can do to cattle. Since the major vectors in this region are dogs, a specific survey has been conducted on this population. This study provides both quantitative and qualitative information. An estimation of the total number of dogs gives the number of dogs in the whole NCD. The estimated vaccination rate is 12 per cent, which is not sufficient to allow control of the disease. The qualitative information is on one hand the relationship between dogs and humans. It appears that very few dogs are restricted, most of them being permitted to roam in search for food. As far as the stray dog population is concerned, the study demonstrates that in fact most of them have owners. As a consequence, the main thing to do for the control of rabies in NCD is to increase the vaccination rate of owned dogs. This can only be done through a better information campaign, so that people know where to go and are aware that they must immunise.

INTRODUCTION

North Central Division is a region where rabies is a growing problem. It is a big danger for human health, causing 15 to 20 deaths in horrible pain each year, but it also has an economic impact since it can affect cattle and cause big damages in the stock. The dog is the main vector and reservoir of this disease, but wild animals such as jackals play an important role in the persistence of the disease because they are often linked with canine rabies. Many authors (WHO/WSPA, 1990; Perry, 1993) have argued convincingly that dog ecology data are required for planning rabies control. Studies on dog populations have been conducted in other African countries (Butler, 1995; Kitala, 1995; Dlamini, 1999), but till now no precise data were available in Namibia. So far, a questionnaire survey was conducted in this region, aiming at providing quantitative information on the dog population and qualitative information on the status of these dogs and their relationship with humans. It also gave an idea of the impact of the current rabies vaccination.

These results give a better idea of the constitution of the population of dogs and of the role they play in the transmission of rabies. The conclusions of this study will be used to adapt measures in the management of the dog population and the vaccination methods to launch against rabies in NCD.

MATERIALS AND METHODS

Questionnaire survey

A homestead questionnaire was designed comprising three parts. The first was the identification of the questionnaire and the location of the people interviewed; the second part contained questions about owned dogs; and the last part was specifically dedicated to the stray dog population. People were asked the number, age and vaccination status of their dogs, the reason for keeping them, the methods of feeding, if the dogs were restricted or not. Concerning the stray dog population, the information asked was about the presence of roaming dogs around their homestead (number, known or unknown) and their attitude towards them.

The homesteads were selected systematically, radiating from the centre of the village sampled, counting two homesteads for higher density areas and one homestead for low density areas. For the towns, the houses surveyed came from different settlement areas (rich, average and poor) and one out of three houses for each area was chosen. In each village or town a mean of twenty questionnaires were collected.

Villages and towns surveyed

Dog densities are closely linked to human densities (Butler, 1995). Based on that, the territory of NCD was geographically divided into three areas with decreasing human densities. One is composed of towns only, the other of rural areas with above 45 inhabitants per square kilometre and the last one of rural areas with under 45 inhabitants per square kilometre. This defined the three groups of our study in which villages and towns were sampled. Four towns out of 11 were chosen at random for group one. These were Ondangwa, Omuthiya, Ruacana and Tsandi. For group 2 and 3, the numbers of villages chosen were proportional to the size of the population of each group, with 19 villages in group 2 and nine villages in group 3. They were also selected at random from a static file so that they were distributed throughout NCD.

Information of people

A talk show was organised on NBC oshivambo service in Oshakati to inform people of the study to be conducted in their areas. The reason for organising the talk-show was to explain to people the aims and objectives of the study, when it would start, and when it would end, what the study would hold for them in the future so that they would be aware and co-operative when practical field work began.

RESULTS

Household data

572 questionnaires were collected out of a total of 84 767 homesteads in the whole NCD (Table 1). Nearly 80 per cent of rural homesteads have dogs, but only 47,8 per cent in the towns; 37 per cent of rural people have two dogs or more, compared with only 22 per cent in towns. The mean of dog per homestead is 0,8 in the towns while it reaches 1,4 and 1,5 in rural areas with high and low density respectively.

Table 1. General data and number of owned dogs

	Group 1 (Urban)	*Group 2 (Big rural)*	*Group 3 (Small rural)*	*Total*
Nb questionnaires collected	46	350	176	572
Total nb of homesteads	5711	54318	24738	84767
% homestead with dog	47.8%	82.3%	77.8%	78.1%
% people with 2 dogs or >	22%	38%	39%	37%
Nb dogs per homestead	0.8	1.4	1.5	1.3
Mean age (years)	1.6	1.86	2.06	1.93

Estimation of the number of dogs

Making a ratio between the number of homesteads interviewed and the total number of homestead in each group, we have extrapolated the number of dogs censused to the whole population of dogs. This gives an estimation of the quantitative importance of dogs in NCD that only includes the owned dogs. The number estimated is 115 000 dogs (Table2).

Table 2. Estimation of the total number of dogs in NCD

	Group 1 (Urban)	*Group 2 (Big rural)*	*Group 3 (Small rural)*	
NB of dogs censused	38	478	255	
NB of homesteads interviewed	46	350	176	
Total number of homesteads	5 711	54 318	24 738	
Total number of dogs	4 718	74 183	35 983	**114 884**

Vaccination rate

The rate of vaccination estimated in our study is 12 per cent, with variations between the different groups, being higher in group 3 with 15.31 per cent than in groups 1 and 2 with 9.68 per cent and 10.44 per cent respectively.

The main reason for not being vaccinated is because the dog is not old enough for vaccination (three months or less), which is the case in nearly one out of four dogs, or was not old enough during the vaccination campaign in the area.

Another important reason why dogs are not vaccinated (42.3 per cent) is that people are not aware where to go. There is no significant difference between the groups but for groups 2 and 3 it means that even if they know where the crushpens are, they don't know that they can get their dogs vaccinated there.

Some people said that they didn't find the time to take the dog. There were twice as many of these people in the urban (22.9 per cent) areas than in the rural ones (11.5 per cent).

Only a few people didn't know that dogs had to be vaccinated. This was around ten per cent in the towns and high-density rural areas, but we noticed that nobody gave that reason in group 3, also the group with the highest rate of vaccination.

One other reason given was that the dog is aggressive or doesn't want to follow the people to the crushpen (six per cent). In rural areas, five per cent stated that they missed the vaccination campaign, either because they didn't know or had misunderstood the date, or they were not at home those days, or they could not go to the crushpen because it coincided with pension day. Finally, in group 3, 5.4 per cent of the people also said that the DVS didn't turn up at their crushpen. In this group 6.3 per cent complained that when they went to the DVS office or to the crushpen with their dog, the DVS had no more vaccine.

Dog-human relationships

Among the 78 per cent of people who do have dogs, they almost all said they had them for protection. Only 4.5 per cent in group 1 and 0.3 per cent in group 2 keep dogs for mere pleasure (Table 3). They are also a source of meat for 33.3 per cent of people, more in rural areas than in towns. In rural areas the dog is also used for hunting game, but only in two per cent of cases.

Table 3. Reasons for keeping dogs

	Group 1 (Urban)	Group 2 (Big rural)	Group 3 (Small rural)	Total
Protection only	81.8%	76.4%	35.5%	64.1%
Protection and eating	13.6%	21.2%	61.6%	33.3%
Protection and hunting	0.0%	2.1%	2.9%	2.2%
Other reason (pleasure)	4.5%	0.3%	0.0%	0.4%

Most of the dogs that eat special food are in towns, but even there this is the case in only 18 per cent of them (Table 4). More often, 80 per cent in group 1 and 96 per cent in group 2 and 3, dogs only eat leftovers, which is porridge most of the time.

Table 4. Method for feeding dogs

	Group 1 (Urban)	Group 2 (Big rural)	Group 3 (Small rural)	Total
Special food*	18.2%	3.8%	3.6%	4.5%
Left over	81.8%	96.2%	96.4%	95.5%

**The dogs which received rich food as milk or meat were included in this case*

Although nearly 15 per cent of the dogs are closed up or tied up all day in town, this only represents three per cent of the dogs in the entire study. The 97 per cent

that are unrestricted represent so far 110 000 dogs of the 115 000 total dogs estimated in the whole of NCR, i.e. 110 000 dogs that can roam day and night.

Status of dogs

In rural areas, nearly all the dogs that are seen roaming around are recognised as being a neighbour's dog (100 per cent in group 3 and 96 per cent of group 2). In towns the results are different and people say they don't know approximately one dog out of two. People were also asked the number of these dogs they saw roaming around their homestead, a number which decreases with the population being, in the mean, 4.5 per cent for the towns, 3.4 per cent for high density rural areas and 2.7 per cent in rural areas with low density.

Attitude of people towards stray dogs

In rural areas 61 per cent of people do nothing when they see a roaming dog because they say they know it; they add that if they didn't know it, they would chase it away. There are only 28 per cent who do nothing in towns and 65 per cent who chase them away. Some townspeople feed them (6.5 per cent).

The people in towns are more preoccupied by the number of roaming dogs – 74 per cent of them consider it a big problem. In rural areas the number of people who care about this problem increases with population density. They compose 51 per cent in group 2, but only 31 per cent in group 3.

The preoccupation of people in towns is due to their fear of being bitten as much as the fear of rabies. The third most popular reason given is that they steal. In rural areas the people are more worried about rabies – more than 55 per cent of them say that is why they consider stray dogs a problem. There are also many of them (35 per cent in group 2 and 43 per cent in group 3) who first claim that dogs come and steal in their homestead.

Only two per cent say the main problem is that they chase cattle or chickens.

Proposals of solutions

Concerning the tie-up order, only two per cent are opposed to such a measure. Some 43.5 per cent of people in towns and only 14.9 per cent in group 2 and 5.7 per cent in group 3 offered some solutions: dogs could be captured or killed, the owners should enclose or tie up their dogs, all the dogs should be vaccinated, dogs should be given enough food, and people given better information on vaccination and availability of the vaccines. The proportion for each proposition is detailed in Table 6.

The figures show that in towns 70 per cent of people proposed to capture or kill the dog while 12 per cent in rural area of high human density and 0 per cent in low human density area. In these two latest groups, 46.2 per cent in group 2 and 90 per cent in group 3 said that all dogs should be vaccinated

Table 6. Proposals from people to solve the problem of stray dogs

	Group 1 (Urban)	Group 2 (Big rural)	Group 3 (Small rural)	Total
Captured	45%	9.6%	0%	17%
Killed	25%	1.9%	0%	7%
Tied up/enclosed	30%	19.2%	10%	21%
All dogs vaccinated	0%	46.2%	90%	40%
Dogs should be given enough food	0%	17.3%	0%	11%
Better information/caccine availability	0%	5.8%	0%	4%

DISCUSSION

In NCD, the dog is a common domestic animal and almost every homestead has at least one. They are considered useful as watchdogs for homestead and property. As a consequence dogs are often aggressive with strangers or even with neighbours. That is why there are many cases of bites.

Dogs are also a significant source of meat, and the percentage estimated in this study could in fact be higher because people are often reluctant to admit to consuming dog meat. In an informal discussion with people in NCD, they alleged that the percentage could be above 60 per cent of the total population. The reason for slaughtering the dog can be a death in the household, if the dogs has bitten someone, or if the dog is ill. Some people even say they kill and eat rabid dogs when they see them. Handling such meat is risky, especially if they have cuts or if the fluids of such animal enter the penetrable mucosa. This is a most unfortunate mode of transmission that leads to human cases with an unknown incidence of bites.

Hunting with the dogs is something of the past in NCD. With the introduction of nature conservation to regulate the illegal killing of wild animals, people rarely hunt.

Despite their value, dogs in NCD are largely unsupervised. Although their owners feed them regularly, they are given low quality protein food, normally leftovers or porridge. Moreover it often happens that people who have many dogs don't give them enough food. As a consequence, dogs suffer from malnutrition. To supplement their poor diet, the dogs have to scavenge. That is why people often complain of dogs that come and steal in their homestead during the day or during the night. It is also why when they are asked for propositions to solve the problem of stray dogs, people say that dogs should be given enough food. This reason is the most important one to explain why there are so many dogs roaming. Moreover, they can sometimes walk long distances to town, for example, drawn by the smell of meat. This number of dogs could be reduced if they were restricted, either tied up or enclosed, but very few owners do this. In towns some are enclosed to guard the house. In rural areas they are only tied up or enclosed when they are very aggressive or when they chase the chickens.

The estimation given in this study of 115 000 only takes into account the population of owned dogs, which make up the majority of the roaming dogs.

However, this figure could be an underestimation since it was calculated with respect to the size of the human population in 1998 (which is likely to have increased). This number is rather high and completely different from the one obtained by the census of dogs at the crushpen, which was around 30 000 dogs. It is, however, close to the estimated number of 140 000 obtained in a survey made in NCD in 2000. The people interviewed in this study were the farmers who had brought their cattle to the crushpen, among whom the proportion of dog owners might be higher than in the population as a whole (which could explain why this last number is bigger than the one in our study. Finally, the estimated size of the dog population is far bigger than the one estimated in 1992, which was 80 000 for the whole of Namibia (Depner, 1992). Since no information is given on the reliability of this last data, we can only assume that the dog population has increased a lot in the past ten years.

The mean age of the dogs is rather low and indicates that the life expectancy is not very high. Among the reasons, one is that dogs receive little veterinary attention. Moreover it is not uncommon to hear that people have eaten the dog because it was ill.

Together with the high percentage of dogs under three months, this low mean also indicates that the turn-over is high in dogs. This is an important criteria to consider since it affects the impact of vaccination. Indeed, it was shown that if the turn-over was too high, an annual vaccination campaign cannot allow the recommended rate of 70 per cent (WHO, 1984).

Moreover, the current vaccination rate is surprisingly higher in rural areas than in towns, and even higher in low-density areas. Such a phenomenon may be explained by the fact that people are already used to bringing their cattle for vaccination and so are more inclined to bringing their dogs.

Foggin (1988) proposed that a sustained vaccination coverage of 50 per cent of dogs would be sufficient to keep rabies to an acceptable and possibly controlled level. But the global rate of 12 per cent, it is still too low to allow a control of rabies.

Significant numbers of dogs are under three months, or were under three months during the vaccination campaign for rural areas. Puppies are often held to be responsible for human cases of rabies, above all in children. So, ideally, they should be vaccinated as soon as they are old enough. If they miss the vaccination campaign, people can bring their dogs to the DVS office, but this cannot be done in remote areas. A bi-annual vaccination campaign would be more efficient but is difficult to establish because of the cost.

Concerning the people who say they don't know where to go, if they are in town, they are unaware that they can go to the DVS office and get their dog vaccinated free of charge. And in rural areas, it means that people don't know that they can get their dog vaccinated at the crushpen. Even people who habitually bring their cattle there during the vaccination campaign said that they were not aware of it. A targeted information campaign should solve this problem.

The people who just didn't take the time do know about the vaccination,

where to go and that it is free of charge, but don't attach real importance to it. To them, the importance of the act of vaccination and the role it plays in the fight against rabies must be clearly presented so that they are persuaded to do it.

Among those who said they had an aggressive dog, some were concerned because they really wanted to get their dog vaccinated but they didn't know how they could. It doesn't represent a big percentage of dogs but shouldn't be neglected because aggressive dogs may be more exposed to the disease than other dogs. Specific measures can be taken for these dogs, using tranquilisation with tablets for example. But this solution, though easy to apply in towns where vets are available, seldom suits the rural areas. In these cases, non-vet staff could be allowed to use these tablets for vaccinating aggressive animals.

In rural areas it sometimes happens that people miss the day of vaccination, either because they didn't know or had misunderstood the date or were not at home on those days, or because they could not go to the crushpen because it coincided with the old age pension. Except by strengthening the information campaign, it is difficult to overcome this since it depends on the people themselves.

When the DVS staff didn't turn up at the crushpen, it transpired that it was possible that people were waiting at one crushpen while DVS staff were at another one nearby or that the date was changed because of rains. Some people also expected them to come to their personal crushpen but did not allow other people to bring their cattle.

Some of the DVS offices are new and don't have power, or have power but don't have fridges to store the vaccines. That is why they cannot vaccinate dogs against rabies.

In rural areas, the dogs that are seen roaming around are almost all well known unlike in the towns, where people know fewer people and so don't know each other's dogs. In rural areas people often know the entire village. The towns also concentrate food with markets and butchers that attract dogs from rural areas. In little towns like Omuthiya for example, there are many dogs roaming in the street, but most of them are known to belong to a nearby homestead. Dogs may also follow their owners when they go to town.

So virtually all the dogs in NCD go roaming with the exception of the puppies. This has a role to play in the transmission of the disease because of the movement and contact with other dogs from another area or with wild animals that could have the disease. In rural areas the number of roaming dogs is in relation to the location of the homestead. The people whose homesteads are close to a water tap, cuca shop or pathway will see more dogs because they follow their owners when they go to those places.

The attitudes of people towards roaming dogs are different in towns and in rural areas. This may be because the number of dogs is bigger in town and that fewer people know these dogs than in rural areas. Rural people care less about these dogs because they know them. Nevertheless, some people also do nothing when they don't know the dog because they are afraid of being bitten and above

all because the dog could be rabid.

The tie up order was one of the solutions found to the problem of stray dogs and to increase the incidence of vaccination. Such measures have already been taken in Rundu and seem to have worked rather well. It is interesting that 98 per cent of the population agreed with this measure. The two per cent opposing said that all stray dogs in fact had owners, so they cannot be shot down like that.

That proposals made by people in the towns are different to those in rural areas is due to the bigger numbers of unknown dogs in towns. Indeed there are many in towns that want them shot or captured, while there are very few in rural areas who agree. The proposals given in rural areas reveal that they don't consider stray dogs as a problem by itself since they have owners. The real problem for them is that they are not vaccinated so they are a danger for people because they can carry rabies. For some it is also because they go roaming so owners should tie up or enclose them or give them enough to eat so they don't go and steal elsewhere.

CONCLUSION

Since the first recorded rabies cases in Namibia in 1887, the disease has caused losses among cattle and small stock and continues to represent a major health hazard in the densely populated North Districts. The dog population of dogs is mostly composed of owned dogs that are left unrestricted and go roaming in search for food to supplement their low quality food. This plays a major role in the spread of the disease.

The current prevention against this disease in NCD is the free vaccination of dogs at crushpens or DVS offices, which only reaches 12 per cent of dogs vaccinated. This rate is not sufficient to allow a control of the disease and has to be improved.

As suggested in a WHO report (WHO, 1988), an efficient program of surveillance and control of rabies will include intersectoral co-operation and community participation. The information must be widely spread, through radio programs, and posters in public and work places. Co-operation with hospitals could also be useful in the tracing of cases. Specific measures aimed at reducing the high numbers in the streets can be taken in towns, such as dog restriction obligations for the owners or tie-up orders.

A better awareness of the high risk population that are kids should also be settled so that they would be careful with stray dogs and would know what to do when facing to a suspect dog. Children, who are high risk, should also be made more aware of being careful with stray dogs and taught what to do when facing a suspect dog.

To reach the recommended rate of 70 per cent of dogs vaccinated, some relevant limits will have to be considered. First, the high turnover of the dog population may not allow such a percentage to be achieved with an annual vaccination campaign. Then, the uncontrolled entrance of dogs from Angola remains a

threat even if the local population of dogs is well vaccinated.

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A SURVEY OF FARMERS' KNOWLEDGE ABOUT EXTERNAL AND INTERNAL PARASITES OF CATTLE ON COMMUNAL GRAZING

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SUMMARY

The main aim of agriculture should be to improve productivity. Resource-poor farmers in marginal areas are faced with constraints to achieving this. These include lack of appropriate knowledge and skills. Extension methodology requires that knowledge and skills imparted to farmers should be measureable in terms of improving productivity as well as improving knowledge levels.

This study was undertaken in Moretele District using a structured interview with 90 farmers as well as assessing their cattle for the control of internal and external parasites. External parasites were evaluated by doing tick counts on five sentinal animals per herd and internal parasites were measured by evaluating pooled samples of faeces for nematode eggs monthly. The data was compared before and after extension to evaluate whether extension had an impact on parasite control. Knowledge was measured by personal interviews before and after implementing a farm visit and training method of extension.

Prior to extension, only 53 per cent of farmers said they knew about ticks and tick diseases. Of those that said they knew, less than a third could recognise ticks correctly or knew which diseases they caused. Only 35 per cent said they knew about internal parasites, however, when questioned about worms, less than one fifth of these farmers actually had knowledge. After extension, there was a marked improvement in the knowledge level. However, there was no appreciable change in the level of internal and external parasites.

INTRODUCTION

Casey and Maree (1993) assert that livestock production is changing from a rural occupation to a highly specialised industry. As social and economic reforms progress, a transition from subsistence to commercial agriculture will gain momentum and small uneconomic units will be consolidated into viable units by virtue of market forces, if not by government policies. The planning of animal production systems to achieve optimum returns, requires a full understanding of the biological principles influencing animal production efficiency, as well as socio-economic situations and aspirations (Bembridge, 1991; Duvel, 1999).

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The main goal of agricultural development should be to increase sustainable farming productivity and welfare of the farming communities and stakeholders. However, resource-poor farmers living in marginal areas are faced with unfavourable soils and climate, a lack of credit, under-developed or non-existent markets, irrelevant research and subsequent lack of adoption of relevant available technologies (Casey and Maree, 1993).

In the past, research has been traditionally carried out in controlled environments that were completely different from those of the users. The results were then given to the farmers for implementation. The research organizations were neither directly responsive nor accountable to the farmers.There is an increasing need for consideration of the entire farming system, more involvement of the farmers in the problem identification and testing of the new technology. In order to address these problems and to make research more relevant, farmers must be involved in all attempts of overcoming problems related to farming (Biggs *et al*, 1991).

The state veterinary services are involved in various extension campaigns to farmers throughout South Africa. During these sessions state employees presumably impart knowledge and skills to farmers on various aspects of farming. There is no state-supported unbiased and effective monitoring of the effectiveness of agricultural extension service. With regard to veterinary extension there are expenses in the form of subsidized vehicles purchase and maintenance, remuneration, equipment, refresher courses, and other allowances (North West Province, 1997).

Botha and his colleagues (2000) reported the following in their research regarding implications for veterinary training and research at Moretele:

- That there is a demand by farmers for visual and written extension material;
- That farmers wanted extension to be conducted to them using their herds;
- That there were infrastructure limitation in conducting extension and that this problem be addressed by the stakeholders collectively;
- That cattle and poultry were the most important animal species and should be the focus points of extension, but the need to curb zoonotic diseases should not be disregarded.

In this regard veterinary clinics, private veterinarians and other role-players should be used in partnership with extension workers. Ticks and tick-borne diseases are of considerable economic significance in South Africa (Bigalke, 1980). The following disorders are associated with tick infestation: blood loss, decreased weight gain (Taylor and Plumb, 1981); damage to hides, teats, udders, prepuce, testes, and ears; toxicity such as sweating sickness, and transmission of tick-borne diseases (Asselbergs, and Lopes Pereira, 1989; Coetzer *et al*,1994; de Vos *et al*, 1983; du Plessis *et al*, 1992; Groocock *et al*, 1988).

The state veterinary extension service delivery is one of the more important community-educational services rendered by the government. State animal health personnel frequently undertake cattle tick and internal worm control extension campaigns, but an evaluation of the farmers' implementation of such advice has

not been monitored and documented in South African research journals to date. This is the motivation for the current investigation into whether veterinary extension service advice is being effectively implemented by farmers.

MATERIALS AND METHODS

In order to gain ample knowledge on the production parameters and mortality patterns in cattle herds, an on-farm survey is imperative since the information thus gathered will help in the designing of better extension messages (Biggs *et al*, 1991). This survey was undertaken in Moretele District using a structured interview with 90 farmers as well as assessing their cattle for the control of internal and external parasites.

Two stage cluster sampling (Thrusfield, 1986) was done where farmers were the primary units and the cattle were the secondary units. Non-random selection criteria (purposive selection) was done using the following criteria:

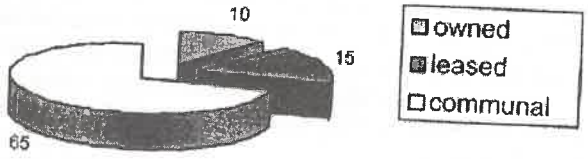
- Thirty farmers with more than five cattle each were selected randomly from three districts of Moretele district of North West Province.
- Five cattle were randomly selected from each herd and used as sentinel animals.

External parasites were evaluated by doing tick counts on five sentinel animals per herd according to the method described by Kiwanuka *et al* (1995) and modified after Spickett *et al* (1990). Removal of feeding adult ticks during sampling was done carefully with a forceps from various sites of the cattle to avoid crushing, and they were placed into a bottle of 70 per cent ethanol (Tice, 1995). Site and animal specifications were made on the specimen bottle before returning the samples to the laboratory for tick identification using a stereoscopic microscope (Tice, 1995). The extension officers also collected faecal samples from the same five sentinel cattle from which tick samples were collected, mixed them, and made a pooled sample that they put into plastic medicine packages. These samples were placed a the cooler box with ice packs and were delivered to the Onderstepoort Veterinary Research Institute for egg per gram (epg) determination (Reinecke, 1989). The data was compared before and after extension to evaluate whether extension had an impact on parasite control. Knowledge was measured by personal interviews before and after implementing a farmer's day and a farm visit and training method of extension by animal health technicians (Bembridge, 1991; Benor *et al*, 1984).

RESULTS

Land use by farmers is shown in Figure 1. Most of the farmers were full time subsistence farmers, i.e. they had no other employment, although they may have had income from other sources, such as pensions. Commercial farmers had their main income derived from farming. Subsistence farmers were defined as small-scale farmers whose income from farming was not the main income. The fre-

Figure 1. **Land use by farmers (n = 90)**



quencies are shown in Table 1.

Table 1. Participation in farming by farmers (n = 90)

Type of participation in farming	*Number of farmers*
Full time commercial	8
Part time commercial	6
Full time subsistence	60
Part time subsistence	16

Eighty-nine (89) of the respondents' farmers practiced extensive beef farming with cross breeds of mixtures of Afrikaners, Brahmans, Ngunis, Jerseys and Frieslands. One herd of Brown Swiss cattle breed was kept for the production of milk and meat. In order to judge whether farmers wished to upgrade their level of farming so that it can bring in some income, farmers were asked about their vision for the future of their cattle farming. The results are shown in Table 2.

Table 2. Farmers' vision for the future of their cattle farming (n = 90)

Vision	*Number of farmers*
To become a commercial farmer	49
To retire from farming	3
To change to crop farming	0
To remain as I am	8
No vision - the government does not want to help us	1
Cannot answer this	10
To have more cattle	10
To survive and support myself and my family	9

Although more than half the farmers see a future where it is possible for them to expand into commercial farming, a large proportion of these farmers do not. This group could be considered to be emerging farmers – farmers that would become commercial if it were not for constraints such as the previous history. This may be very important when looking at whether the farmers will respond to extension messages. None of the farmers want to become crop farmers – they feel that crops are more easily stolen than animals.

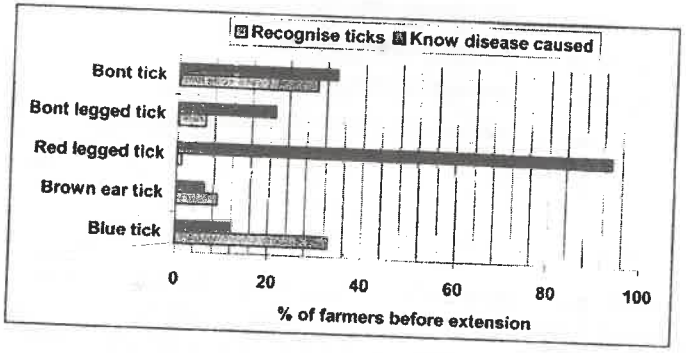
workers who have said that in traditional cattle keeping practices, the number of cattle is more important than their commercial value (*Maree & Casey 1993*). There were some farmers (n=9) who have no ambition to become rich, they only wanted to maintain food security for themselves and their households. These farmers only wanted 'more cattle' so as to secure the survival of the household members. It is quite sad that 11 of the farmers had no vision – one said that the vision is the responsibility of the government and ten could not answer this question.

An inquiry was also made regarding the sizes of the farming land all farmers on the communal land confessed that they do not know the sizes of their farming land and they gave arbitrary figures ('guestimates'). Farmers on owned land and leased land had title deeds and lease contracts respectively that showed sizes of the land; sizes ranged from ten hectors to 2 546 hectors.

EVALUATION OF RESPONDANTS' KNOWLEDGE OF CATTLE TICKS, TICK-BORNE DISEASES AND TICK CONTROL

Before the extension campaign, all 90 farmers were asked if they knew the ticks that transmitted diseases or caused other disorders and 53 per cent acknowledged that they knew while 47 per cent said that they did not know. Figure 2 depicts the answers, of the 53 per cent that answered the question, about the description of the tick and the diseases or disorders caused.

Figure 2. **Percentage of farmers that correctly identified ticks and knew which diseases they caused before extension**



The deduction from the above figure is that what the farmer claims to know is not necessarily a reflection of what he does know. Eight of the farmers (n=90) did not dip their cattle. The rest dipped their cattle as shown in Table 3. From this table it can be seen that although farmers were using different types of dipping methods, the favoured method seemed to be cattle hand spraying with a liquid mixture of dip and water. All the respondents claimed to be using the

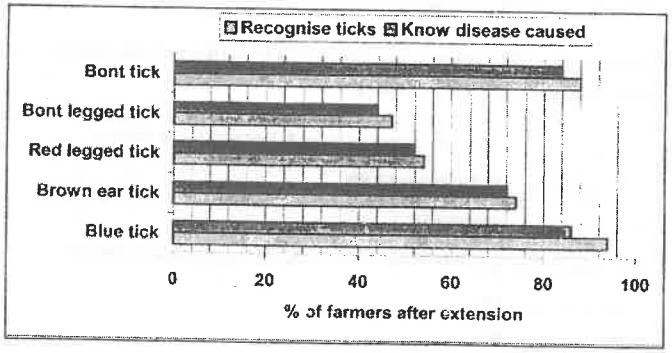
right dilution according to the instructions on the leaflet found with the dip or obtained from the sales personnel.

Table 3. **Types of dipping methods used by respondents**

Dipping method	*Number of farmers*
Plunge dip	2
Spray race	5
Hand spray	61
Hand dress	10
Pour on	21
Injection	3
Acaricide tags	0

The farmers were again questioned following a farmers' day and visits to their farms by animal health technicians. The results are shown in Figure 3. It is obvious that their knowledge of ticks and the diseases they cause had significantly increased.

Figure 3. Percentage of farmers that correctly identified ticks and knew which diseases they caused after extension



The following different adult ticks species were collected per predilection site during the period March 2001 to May 2001 from five (5) cattle by the Moretele animal health technicians, according to the procedure defined by Tice in 1995. The samples were taken to the Onderstepoort Veterinary Research Institute for identification (Spickett 1999-2000).They were identified as follows:

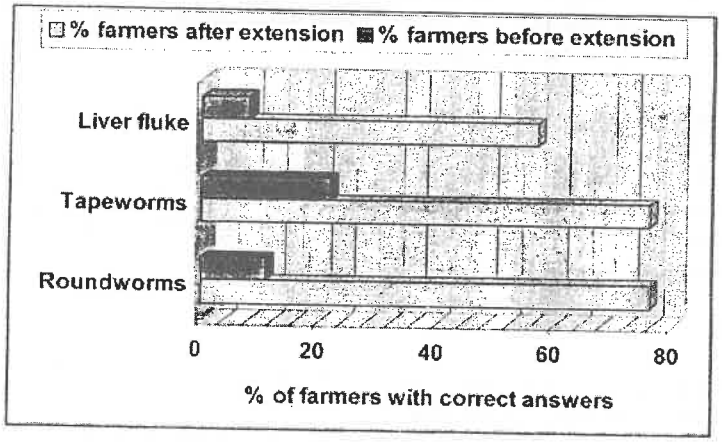
1. *Boophilus decolaratus*,
2. *Amblyomma hebraeum*,
3. *Hyalomma truncatum*,
4. *Rhipicephalus appendiculatus*,
5. *Rhipicephalus evertsi evertsi*.

There was no significant difference in levels between the three areas investigated (Personal communication, Spickett 2002). *Hyalomma truncatum* was less prevalent than the rest of the ticks species identified (0 to 1 adult tick per animal). It was also found that the average cattle tick burden was mild (2 to 12 adult ticks per animal) during this season of the year (Baker & Ducasse 1967; Londt *et al* 1979). There was no significant difference between the levels before and after extension (personal communication, Spickett 2002).

EVALUATION OF RESPONDENTS KNOWLEDGE ABOUT CATTLE WORMS

All 90 farmers were asked if they knew about cattle worms and only 35 per cent of them said that they know about them while 65 per cent said that they did not. Figure 4 shows the farmers' level of knowledge of worms and the symptoms they cause before and after extension.

Figure 4. Percentage change in farmers' knowledge before and after extension



The deduction from the above figure is that what the farmer claimed to know about worms was not necessarily correct because when subjected to deeper questioning on the description of the various worms most of them give wrong answers. The 32 farmers were asked if they were de-worming their cattle for internal worms and only 31 per cent of them said that they do de-worm their cattle while 69 per cent of them said that they do not.

The average levels of strongyles and conical fluke determined from the pooled faecal samples were low (strongyles 41-57 epg, conical fluke 23-77 epg). These levels are not considered to be a cause of disease in cattle. There was no significant difference in adult tick levels before and after extension.

CONCLUSIONS

The deductions from the above is that a high percentage of farmers remembered things they were taught about ticks and worms. However not all farmers remember what they were taught and this may be linked to age and educational level.

The relatively low levels of adult ticks are probably the result of endemic stability as described by Tice *et al* (1998) for communal cattle in the neighboring Odi District, North West Province. Clinically there are very few cases of tick borne diseases and damage due to ticks in the cattle.This study found that most of the farmers were dipping their cattle and the levels of ticks support the suggestion by Tice *et al* (1998), that hand spraying and pour-ons are probably as effective and more economic than plunge dips in this area.

In regard to internal parasites, Reinecke (1960) suggests that deworming of adult cattle is not as important as it is in sheep. This is borne out by the average low epg, even although the veld is heavily grazed and the faeces is not removed. Further work may be needed to see if there is an increase in average daily gain of dewormed weaners from herds on communal grazing as described for commercial cattle (Kyvsraad *et al*, 2000).

ACKNOWLEDGEMENTS

Dr A Vatta and Dr A M Spickett of the Onderstepoort Veterinary Institute are thanked for access to preliminary data on the levels of tick and worm infestation of cattle in Moretele District. The National Research Foundation is thanked for funding the research.

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RISK FACTORS ASSOCIATED WITH BELGIAN CATTLE POSITIVE TO BOVINE HERPES VIRUS-1

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SUMMARY

Programs to eradicate bovine herpesvirus 1 (BHV-1) have been implemented in several European countries to facilitate the free trade of cattle, semen and embryos within the European Community. A preparatory step towards the design of an eradication program in Belgium consisted in investigating the BHV-1 prevalence. In 1998 the national BHV-1 seroprevalence (apparent prevalence) in the Belgian cattle population was determined by a large serological survey. A design-based analysis of the survey results of the unvaccinated group of herds (N=309; 11 284 cattle), taking into account the stratification and clustering, indicated that the animal-level seroprevalence was 36 per cent. The median within-herd seroprevalence was estimated to be 33 per cent (quartiles = 14 - 62), whereas the herd-level seroprevalence was 67 per cent.

A logistic random-effects model investigating risk factors for animal seropositivity revealed that purchased cattle had a two-fold increase in odds of being seropositive (odds ratio OR = 2.25) compared to homebred animals; an increase in age of 1 year was associated with OR = 1.6, whereas an increase in herd size of 10 animals was associated with an OR = 1.27. However, significant negative interactions were found between purchase and age (OR = 0.93) and purchase and larger herds (OR = 0.96). No association was detected between the BHV-1 animal-level seroprevalence and the sex of the cattle, nor with the type of herd, nor with the density of cattle or herds in the municipality.

The potential risk factors associated with animal-level BHV-1 infections (true prevalence) were investigated by examining the impact of the test misclassification probability on the seroprevalence risk factor analysis. This was done via a grid-analysis based on expert opinion regarding the imperfect diagnostic test characteristics. Due to high computational runtimes, no final conclusions could yet been drawn regarding these true prevalence risk factors.

INTRODUCTION

Infectious bovine rhinotracheitis is caused by the bovine herpesvirus type I (BHV-1). It is an enzootic disease on the B List of the Office International des Epizooties (O.I.E.). Programs to eradicate BHV-1 have been implemented in

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several European countries to facilitate the free trade of cattle, semen and embryos within the European Community. A preparatory step towards the design of an eradication program in Belgium consisted in investigating the BHV-1 prevalence. The bovine herpesvirus-1 (BHV-1) seroprevalence (apparent prevalence) in the Belgian cattle population was determined by a large serological survey that was conducted from December 1997 to March 1998, as reported by Boelaert *et al* (2000). The survey had a stratified one-stage cluster sampling design. Blood samples were tested for the presence of antibodies to glycoprotein B of BHV-1, using a commercially available blocking ELISA (HerdChek®, Idexx, France). No differentiation could be made between vaccinated and infected animals, since the exclusive use of marker vaccines was only imposed by law in 1997 by the Belgian Veterinary Authorities. In 309 unvaccinated herds, the overall herd and median within-herd seroprevalence were estimated to be 67 per cent (95 per cent CI=62-72), and 33 per cent (quartiles = 14-62), respectively. A design-based analysis, taking into account the effects of clustering and stratification estimated the animal-level seroprevalence to be 36 per cent (95 per cent CI=30-42) (Speybroeck *et al*, 2002).

Risk factors associated with seropositive BHV-1 Belgian cattle

There are few risk factor studies regarding seropositive cattle. Without considering the vaccination status of herds, risk factors for the BHV-1 herd apparent prevalence status of all Dutch herds with dairy cattle were investigated by a census survey in November 1994 by van Wuyckhuise *et al* (1998); they found that larger herdsize, dairy herds with beef/veal animals, and higher density of herds in the municipality were associated with herd seroprevalence. Moreover, an interaction between farm type and purchase of stock was observed. Van Schaik *et al* (1998) analysed data of 107 farms which had been never vaccinated against BHV-1. BHV1-seropositive farms purchased cattle and participated in cattle shows more often compared with BHV1-negative farms. A BHV1-seropositive farm also had more (professional) visitors in the barn who used farm clothing less often. The BHV1-positive farms were found to be situated closer to other cattle farms compared with the BHV1-negative farms.

Risk factors associated with truly infected BHV-1 Belgian cattle

Infectious disease surveys are affected by the nature of the study design (sample or census survey), the study population (subclinical or clinical, vaccinated or unvaccinated), the type of prevalence parameters studied (herd prevalence, individual-animal prevalence or within-herd prevalence), the diagnostic test used, and the age of the tested animals. Comparison of these survey results is difficult. Moreover, few studies adjust the seroprevalence for factors such as diagnostic test sensitivity and specificity to calculate the true prevalence (creating further difficulty in comparison across studies).

Because of limitations in current diagnostic test technology, it has been impossible to devise the perfect test, which would predict the infection status of

subjects with total accuracy. This is also the case for the gB-blocking ELISA. Kramps *et al* (1994) estimated the diagnostic sensitivity of the gB-blocking ELISA to be 99 per cent. The estimated diagnostic specificity of this test in a BHV-1 free and unvaccinated animal population ranges from 96 per cent (Kramps *et al*, 1994) to 99.7 per cent (de Wit *et al*, 1998). However, these test characteristics may vary among subpopulations (Greiner and Gardner, 2000).

Wentink *et al* (1993) reviewed the literature regarding the risk of infection with BHV-1. They concluded that BHV-1 is mainly introduced to cattle herds by cattle in the incubation period or in the acute phase of the infection, and by latently infected cattle. Introduction by semen, aerogenic transmission from neighbouring farms or over even greater distances, and transmission via humans are probably rare events.

The primary objective of this study was to investigate the survey results for possible animal-level risk factors that might be associated with bovine herpesvirus-1 infection in Belgian cattle.

MATERIALS AND METHODS

Statistical investigation of risk factors associated with BHV-1 seropositive Belgian cattle

In the BHV-1 data set, covariates, obtained via SANITEL, the central computerized database for the identification and registration of the Belgian cattle population (Ministry of Small Enterprises, Traders and Agriculture, Belgium), have the following structure. First, we have the herd-level explanatory variables. These are the type (dairy, mixed or beef) and size of the herds (number of cattle on the premises). Secondly there are the animal-level covariates age, sex and origin (purchased or homebred). Lastly, the density of the cattle population, and the density of herds in the municipalities where the herds were located were determined by dividing the number of cattle and herds, respectively, by the amount of effective agricultural land. Data on the amount of agricultural land per municipality were obtained from the Belgian Agricultural Statistics Yearbook, 1997 (National Institute of Statistics, 1997).

A logistic random-effects model approach was used in order to account for herd differences (within-herd seropositivity). Collinearity between the continuous predictor variables was verified by Pearson correlation coefficient. Pearson chi-square was used to verify association amongst categorical predictors. All two-by-two interactions and second-order (quadratic) factors were considered in a forward stepwise-selection procedure, keeping an eye on the (biological) plausibility of the parameter values. Selection of the (relative) most important models was based on Akaike's Information Criterion (Akaike, 1974).

All analyses were performed using the statistical software Stata®, Version 7.0/SE (Stata corporation, USA). The procedure Gllamm with adaptive Gaussian quadrature was implemented. Gllamm, generalized linear and latent mixed models, is a program that fits a large class of multilevel latent variable models

including multilevel generalized linear mixed models. Recently, adaptive quadrature has been proposed for multilevel models (Rabe-Hesketh *et al*, 2002). The number of points for Gauss-Hermite integration of the random effect, was presently set at 30. Gllamm allows a random parameter for the intercept.

Statistical investigation of risk factors associated with truly infected BHV-1 Belgian cattle

The impact of the test misclassification probability on the seroprevalence risk factors was investigated. Briefly, a true prevalence variable was imputed, using expert opinion regarding the imperfect diagnostic test characteristics. Then the final model selected with seroprevalence as response was re-run with true prevalence as new response.

Expert opinion regarding the diagnostic sensitivity and specificity

To investigate the risk factors associated with truly BHV-1 infected Belgian cattle, expert opinion was solicited. Ten experts expressed their current belief and uncertainty about the diagnostic characteristics of the used BHV-1 gB-blocking ELISA (generation of 1998, Idexx® ELISA test). Experts were selected from the O.I.E. reference laboratoria for BHV-1 together with veterinary epidemiologists with a doctoral thesis on BHV-1 epidemiology. They were contacted by electronic mail only. The main objective was the investigation of the age-related diagnostic sensitivity and specificity of this test in an unvaccinated cattle population. The gB-blocking ELISA is considered as the most sensitive test to detect serum antibodies to BHV-1. For the purpose of quantifying beliefs, a trial roulette form was circulated as developed by Gore (Gore *et al*, 1987a, b and c; Gore in Errington *et al*, 1991).

Grid analysis

A grid analysis was implemented using a Stata® ado-file. Via this program the dataset was transformed a number of times according to the range of values obtained by the expert opinion for the diagnostic sensitivity and specificity. These characteristics are used to impute a 'truly infected' binary response. Different true prevalences were assumed to be able to create this latter variable, via the predictive values. Via gridanalysis, a sensitivity analysis was carried out towards the impact of the expert opinion figures on the seroprevalence risk factor analysis.

RESULTS

Descriptive data exploration

The median herd size of the herds was nineteen overall and 60 for dairy herds, 59 for mixed herds and seven for beef herds. These variables were right-skewed (plots not shown). The density variables were less skewed. The average age (in years) of the animals was lowest in beef herds (2.8), higher for mixed herds (2.9) and highest for dairy herds (3.1).

No collinearity was discerned between the continuous covariates. Sex and purchase were associated (Pearson chi-square(1) = 39.3865, $p < 0.001$): one in four cows are purchased compared to one in three bulls.

Table 1 reports the animal-level seroprevalences. The confidence intervals overlap, which make the point estimators the same for the three types of herds.

Table 1. National seroprevalence of bovine herpesvirus-1, per herd type, in Belgium, 1998

Type of herd	*Animal-level seroprevalence*	
	Number tested and validated (*)	*seroprevalence (%) [95% CI]*
Overall	11 284	36.1 [30.1-42.1]
Dairy	4 152	34.6 [25.2-44.1]
Mixed	3 709	42.2 [31.2-53.4]
Beef	3 387	31.1 [21.0-41.2]

(*): There were 36 missing response values

Visual inspection of contingency tables (not shown) indicated that seroprevalence increased with herd size up to a certain limit. This motivated explorative modeling with inclusion of quadratic terms. There were no obvious differences between the seroprevalences of cows and bulls, but the OR of purchase (when corrected for herd effects) was significantly larger than unity. Seroprevalence increased with age, but again with a hint of a non-linear effect, again prompting the inclusion of a quadratic term.

Statistical investigation of risk factors associated with BHV-1 seropositive Belgian cattle

The final logistic random-effects model investigating risk factors for animal seropositivity revealed that purchased animals, older animals and larger herds were associated with an increase in odds of an animal being seropositive. However, the interactions between these risk factors were also significant (Table 2). No association was detected between the BHV-1 animal-level seroprevalence and the sex of the cattle, nor with the type of herd or the density of cattle or herds in the municipality.

Second-order or quadratic effects of herd size and age were checked, as suggested by the descriptive exploration. However, considerable multi-collinearity remained present, even after having centred those predictor variables. Consequently, polynomial regression was abandoned.
The final model at this point is:

Purchase + age + herdsize + herdsize*purchase + age *purchase

Table 2. Final logistic random-effects regression model of risk factors for cattle seropositive to bovine herpesvirus-1, in Belgium, 1998

Risk Factor	*odds ratio*	*SE* [a]	*95% confidence interval*		*p-value*
Purchase (1 = yes, 0 = no)	2.25	0.45	1.52	3.33	< 0.001
Herd size (number of animals on the premises / 10)	1.23	0.05	1.13	1.33	< 0.001
Age (years)	1.60	0.03	1.54	1.67	< 0.001
Interaction Herd sizexPurchase	0.96	0.02	0.93	0.99	0.010
Interaction AgexPurchase	0.93	0.03	0.86	0.99	0.032

[a] *standard error* — *Akaike's Information Criterion = 3310.18*

Statistical investigation of risk factors associated with truly infected BHV-1 Belgian cattle

Table 3 summarizes the results of the expert opinion regarding the epidemiological test characteristics.

Table 3. Current belief about the diagnostic characteristics of the used BHV-1 gB-blocking ELISA (generation of 1998, Idexx(R) ELISA test) expressed by ten experts

		≤ 8 months	8 to≤12 months	1 to≤ 3 years	>3 years
Animal with maternal antibodies	Diagnostic Sensitivity (range)	1	0.80 - 0.995	0.965 - 0.995	0.975 - 0.995
	Diagnostic Specificity (range)	0	0.93 - 0.995	0.93 - 0.995	0.93 - 0.995
Animal without maternal antibodies	Diagnostic Sensitivity (range)	0.96 - 0.995	0.96 - 0.995	0.96 - 0.995	0.96 - 0.995
	Diagnostic Specificity (range)	0.93 - 0.995	0.93 - 0.995	0.93 - 0.995	0.93 - 0.995

Gridanalysis

Still under evaluation.

DISCUSSION

This cross-sectional study measures the disease status of the members of a population at a particular time. The disease patterns indicated only reflect associations, and do not permit causal interpretations about the results obtained. However, for purposes of causal interpretations, cross-sectional studies are suited to studying permanent factors, because such factors cannot be altered by the passage of time or by the presence or absence of disease (Martin *et al*, 1987). Therefore, in this survey, the recorded herd, management and animal-level characteristics are of a permanent type with regard to the lifetime of the cattle.

Statistical investigation of risk factors associated with BHV-1 seropositive Belgian cattle

For uniform dependence structures among clustered observations, the standard approach is to use a mixture distribution based on a random effects model as an artificial construct to generate a multivariate distribution. This requires some form of efficient multidimensional integration or recursive updating. Here we used (logistic) random effects models in order to account for herd differences. The use of a random-effects model approach can be motivated by arguing that animals belonging to a herd share the same environment (physical location), as well as many characteristics due to, e.g., the type of farm (milk- or meat-oriented). These shared factors, whose effects can change from herd to herd, create dependencies between responses observed for the individual animals.

As far as known to the authors, this is one of the first times that maximum-likelihood estimates of the model parameters of a risk factor analysis were computed based on the numerical integration procedure using the adaptive Gaussian quadrature Gllamm macro in Stata®. The use of adaptive Gaussian quadrature for the random effect models was motivated by the fact that different conclusions with respect to significance and signs of the coefficients were reached, depending on the number of integration points used, when adaptive quadrature was not employed.

The recorded risk factors were examined according to their association with an animal being seropositive to BHV-1. Purchase status of the animals, age of the animals, and herd size seemed to be statistically important predictors for BHV-1 seropositivity of an animal.

We found a striking association between the purchased status and the animal seroprevalence. This finding agrees with several studies. The purchase of infectious cattle is reported as an important source of BHV-1 introduction (Van Wuyckhuise *et al*, 1998; Van Schaik *et al*, 1998).

Size of the farm and age of the animals also are statistically associated with BHV-1 animal seropositivity. The older an animal, or the larger the farm it belongs to, the higher the chance to show a serological positive result. Both risk factors can be explained from a biological viewpoint. Especially the seroprevalence in dairy herds is often found to be dependent on the age of cattle. Young stock is seronegative, whereas milking cows are more often seropositive (Van Wuyckhuise *et al*, 1993). The risk of BHV-1 transmission among cattle within herds is higher at larger herd size. This may be explained by the (direct) within-herd contact structure. In smaller herds the number of susceptible animals is smaller throughout the year, so infection may not be sustained. Larger herds usually have loose-housing barns, creating more contact between infected and susceptible animals. These herds possibly also have more visits by animal handlers (farmers, inseminators, veterinarians, traders) (Van Wuyckhuise *et al*, 1998). More visits augment the risk for infection transmission. Also the double-

track survival strategy of herpesviruses in nature sustains the biological explanation of herd size being a risk factor. During primary infection, herpesviruses are disseminated within susceptible populations, which raise strong immune responses and overcome in most cases the diseases associated with the infections. The latent viruses represent a long-term reservoir that becomes meaningful upon reactivation. Then, seemingly healthy animals are able to re-excrete and transmit the virus to non-immune as well as to immune hosts (Thiry *et al*, 1985; Thiry *et al*, 1987; Engels *et al*, 1996).

The analysis of the interaction between these risk factors lead to the observation of a less synergetic effect between the risk factor purchase and older animals, and purchase and bigger herds.

No association was detected between the BHV-1 animal-level seroprevalence and the sex of the cattle, nor with the type of herd or density of cattle or herds in the municipality. At this point we may emphasize that the animal-level seroprevalence was estimated taking into account the stratification and the clustering. This is important, since the calculation of the variance of the seroprevalence using formulae for simple random sampling may yield overly optimistic estimates. It appears that in surveillance studies investigating prevalence of diseases the precision of the prevalence is often overestimated (McDermott *et al*, 1994). Then risk factors would be more easily reach the significance level.

Statistical investigation of risk factors associated with BHV-1 infected Belgian cattle

Additionally, the testing procedure had inherent probabilities of misclassification, due to diagnostic test inaccuracy. If one would know the diagnostic sensitivity and specificity of the test, the true prevalence could be estimated. Unfortunately, the test characteristics are, in general, not known. Moreover, these test characteristics vary among subpopulations (Greiner and Gardner, 2000). The impact of this misclassification on the BHV-1 risk factor analysis is currently being investigated. The grid-analysis that is currently evaluated incorporates expert opinion in a frequentist framework. The aim is to run it five times, and to analyse the pooled results. An analogous analysis could be implemented in a Bayesian framework.

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A PRELIMINARY ASSESSEMENT OF DISPERSAL OF AFRICAN BUFFALO (*Syncerus caffer*) IN THE KRUGER NATIONAL PARK USING MICROSATELLITE MARKERS

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SUMMARY

Bovine tuberculosis (BTB) is an alien invader in the Kruger National Park (KNP) where African buffalo act as efficient maintenance hosts of this pathogen. In recent years spill-over of the infection to other species such as kudu, lion, leopard, cheetah, baboon, genet, and hyaena has caused concern amongst conservationists. It is believed that the disease was introduced to southern KNP in the latter part of the 1950's. Based on results from the 1998 BTB survey, in which > 600 buffalo were randomly sampled throughout the park, infection rates of 1.5 per cent, 16 per cent and 38.2 per cent, were found in the northern, central and southern sections, respectively. Since the spread of a disease and its persistence are functions of dispersal between subpopulations, a genetic analysis of buffalo is being conducted using microsatellite markers, in order to measure patterns of buffalo dispersal within KNP. We report here on preliminary results involving the screening of 90 animals with 10 microsatellite markers. The observed levels of heterozygosity indicate that genetic diversity within KNP is high, despite a recent population bottleneck caused by the rinderpest pandemic in the 1890s. Genetic differentiation amongst herds is however low, indicating that KNP probably consists of a single panmictic population. By increasing both the sample size of buffalo and the number of microsatellite loci it is hoped that an extensive buffalo population study in the KNP will assist with predicting the spread of BTB within this ecosystem.

INTRODUCTION

The Cape buffalo (*Syncerus caffer*) occurs throughout the Kruger National Park (KNP). While never far from water they are at home in a wide array of vegetation types. These animals, as one of the 'big five', attract thousands of tourists to South Africa annually. Outside of National Parks they are highly valued both as hunting trophies and ranching stock. The market value of buffalo is, however, dependant on whether they are pathogen-positive or 'clean'. Buffalo

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are carriers of a host of diseases, including foot-and-mouth disease (FMD), corridor disease (CD) and bovine tuberculosis (BTB), to which they are relatively well adapted. The introduction of livestock diseases has, however, had dire consequences for South Africa's buffalo population. The rinderpest pandemic almost led to the extinction of the species, eradicating approximately 95 per cent of the total population in KNP (Smithers, 1983). Kruger scientists estimate that, although as few as 20 KNP buffalo survived the epidemic (Stevenson-Hamilton, 1911), the population is a robust 30 000 individuals today. BTB, caused by *Mycobacterium bovis*, is an alien microparasite thought to have been introduced to buffalo in the KNP four decades ago, with the first formal diagnosis occurring in 1990. The disease is thought to have been contracted from infected cattle, bordering the southern part of the game park (Bengis *et al*, 1996). In 1998, the prevalence of the disease in herds in southern KNP was more than 38 per cent, with some herds having as many as 90 per cent infected individuals. BTB has subsequently spread northwards and now spills over to numerous other species within and outside the park (Winterbach, 1998). To date, more than 27 species are known to contract the disease, including badger, lion, kudu, rhino, and humans. The disease ultimately threatens not only wildlife populations, but it potentially has serious economic implications for the cattle industry through reverse-transmission from buffalo. Finally, *M. bovis* is very closely related to *M. tuberculosis*, and can infect humans (Daborn, 1995).

A BTB working group has been established to study various aspects of BTB in the complex natural system of the KNP. Dispersal of buffalo is an important part of these investigations because migrating individuals vector the disease among neighbouring herds (Cross and Getz, in review). Currently, Paul Cross is using radio collars and other markings to track movements of individuals among herds, but is limited to assessing the movements of only marked individuals. This analysis can be complemented using molecular genetic techniques to analyse DNA from both marked and unmarked individuals. An assessment of the changes in the frequency of alleles across KNP herds will be invaluable in helping reconstruct parameters used to characterize buffalo migration throughout KNP.

Dispersal in a genetic sense is defined as the distance between an individual's birthplace and that of its offspring (Koenig *et al*, 1996). The mean and the variance of the dispersal distance are important factors in predicting the spread of BTB. Four types of molecular markers are useful tools to establish dispersal, viz. maternally inherited mtDNA markers, paternally inherited Y-chromosome markers, unlinked DNA microsatellite markers and so-called juxtaposed microsatellite loci. In the last decade the use of DNA-microsatellites have contributed immensely to the field of population genetics. DNA-microsatellites are stretches of DNA with a simple repeat motive, mostly from a non-coding part of the genome. The repeat number varies amongst different alleles of a locus. Their high mutation rates make them highly polymorphic which result in a large number of alleles being present in most populations. These markers are inherited in a

simple Mendelian fashion. Hence, the more closely related two individuals are, the more alleles they will share. A range of microsatellites have been developed for gene mapping studies in cattle, and most of these markers have been shown to be variable in buffalo (Van Hooft *et al*, 1999). Large-scale application of bovine microsatellite primers to buffalo should permit assessment of dispersal, population bottlenecks and differentiation and is our ultimate goal. A first step in achieving this is to determine whether any herd differentiation exists and what the level of heterozygosity is within the KNP. With this preliminary aim in mind, buffalo representative of geographically diverse herds throughout the KNP, will be screened with ten unlinked microsatellite markers.

MATERIALS AND METHODS

Samples

The samples used in this study were obtained from the 1998 survey of BTB in the KNP and represent approximately 15 per cent of the total number of buffalo sampled. Nine herds were identified for this study, viz. three from the north, two from the central area and four from the south of the park. Ten individuals from each herd were chosen from the total of 616 animals sampled. In order to minimise the randomising effect of dispersal, predominantly young animals (< 3 years old) that are still resident within their natal herd, were selected for genetic characterisation. Equal numbers of male and females were chosen.

Genetic characterisation and analysis

DNA was extracted from blood samples and a total of ten microsatellite loci were analysed. The loci were amplified, using fluorescence labelled primers, in a multiplex PCR (polymerase chain reaction) and amplification products resolved on an ABI 377 DNA sequencer. GENOTYPER (Applied Biosystems®) was used to score the alleles from each individual. Analysis of genetic diversity was performed with the CERVUS package (Marshall *et al*, 1998). The data was then formatted for the GENETIX software (Belkhir *et al*, 1996) in order to calculate the genetic differentiation between the herds. Furthermore, an assignment test was carried out with the help of GENECLASS (Cornuet *et al*, 1999).

RESULTS

The ten microsatellite loci were in general quite variable, with an average of 6.9 alleles (Range 2-14). The average heterozygosity was 0.637 (Range 0.274-0.832) indicating high genetic diversity. With this complement of loci, the microsatellite data allows for a 99.8 per cent correct total exclusion of parents, provided the genotypes of these are known (CERVUS). Of the 90 buffalo analysed, we obtained a complete data set for 85, in other words all ten loci were scored. Only these 85 individual were used in the subsequent population genetic analysis.

The microsatellite data were analysed for genetic differentiation between herds using the GENETIX program (Belkhir *et al*, 1996). Genetic diversity for buffalo throughout the KNP was moderate (Fit = 0,074), however most of the variability existed within herds (Fis = 0.073), leaving very little differentiation between herds (Fst = 0.0012, n.s. p>0.39). Despite this, an assignment test was still performed using the GENECLASS program (Cornuet *et al*, 1999). The resolving capability of the analysis was not strong because most individuals are assigned to several herds, rather than to a single herd. No animals, however, are excluded from their herd of origin. Of the 85 animals analysed, 75 were assigned to their herd of origin with the highest probability, whilst two individuals were assigned to their herd of origin, to the exclusion of all other possible herds.

DISCUSSION

A preliminary evaluation of the data from the unlinked microsatellites indicates little population structure within KNP. This finding is not unexpected due to the bottleneck following the rinderpest outbreak, which most likely forced the surviving animals into a single herd, eliminating any previous population substructuring and severely reducing heterogeneity. The rapid expansion that followed the rinderpest outbreak probably resulted in a rather random distribution of genetic markers. Furthermore, taking the mobility of buffalos and the size of KNP into consideration, one might not expect to see isolation by distance. Only a single migrating individual per generation will prevent differentiation of otherwise isolated sub-populations (Wright, 1943). In order to assign individuals to their population of origin, a Bayesian approach was followed using the program GENECLASS (Cornuet *et al*, 1999). The program excludes individuals from populations from which they are unlikely to have originated. While these data are not conclusive, the addition of more loci and animals should improve the power of the assignment test to the extent that most individuals will be assigned to their herd of origin with a very high probability and most animals with parents from different herds should be identifiable.

The microsatellite approach detailed here will be complemented by nucleotide sequencing of D-loop mtDNA, which will further assist in determining the extent of the bottleneck caused by rinderpest. Being maternally inherited, it is also expected to yield information on the dispersal of buffalo cows in the KNP and should provide insight into the possible isolation barrier posed by the Olifants river over historical time. Additional microsatellite primers targeting juxtaposed loci and variable Y-chromosome loci in the non-recombining area of the chromosome is in progress. The search for appropriate markers is however challenging because of the lack of extensive pedigree information on KNP buffalo. This essentially means that it is impossible to measure the exact proximity of two or more loci thereby negating unequivocal identification of juxtaposed loci. It is anticipated, however, that bovine genome mapping data can be extrapolated to buffalo, thereby facilitating the search for these markers. The Y-chromosome

markers will supply information on male dispersal, whilst the tightly linked or juxtaposed markers will confirm common ancestry.

We envisage that the data arising from the combined molecular approaches (linked, unlinked and Y-chromosome microsatellite markers and D-loop mtDNA sequences) will provide valuable information for conservation and management of buffalo populations throughout South Africa, particularly because it complements data collected through observations in the field.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Prof. P van Helden (University of Stellenbosch) for supplying the buffalo samples used in this preliminary survey and are grateful to Prof. J du Toit (Mammal Research Institute, University of Pretoria) for valuable discussions. This research was supported in part by the US NSF Ecology of Infectious Disease Grant DEB-0090323 to WMG.

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GENETIC DIFFERENTIATION OF AFRICAN SWINE FEVER VIRUSES OF THE ESAC-WA GENOTYPE

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SUMMARY

African Swine Fever virus is the causative agent of an economically devastating disease of domestic pigs that is characterised by up to 100 per cent morbidity and mortality. As there is presently no vaccine available with which to combat the disease, control is based on zoosanitary measures and slaughter of infected and in-contact animals. Methods that permit rapid diagnosis of the disease and provide information on the most likely source of an outbreak are essential to prevent re-introduction via similar routes of infection. Although some progress has been made with molecular methodology for diagnosing and characterising ASF viruses, present techniques are not able to resolve relationships within a viral genotype comprised of isolates from Europe, South America, the Caribbean and West Africa, the so-called ESAC-WA genotype. Given the importance of the disease in West African in historically in the other afore-mentioned geographical localities, it is essential that alternative molecular methods are investigated which address the perceived lack of heterogeneity within this group of viruses. With this in mind, a hypervariable repeat region located within the 9RL open reading frame (ORF) was targeted for genetic characterisation. For this purpose viruses recovered from outbreaks of the disease in West Africa between 1982 and 2000 were selected, as well as viruses from Europe that are of historical interest. The central variable region (CVR) occurring within the 9RL ORF was targeted with flanking primers. Amplicons of variable size (353-689 bp) were obtained following polymerase chain reaction (PCR) amplification. Products were purified and nucleotide sequences determined prior to phylogenetic analysis based on amino acid alignment of the sequences. Results indicate the presence of 11 discernable CVR lineages, five of which contain viruses of European origin, pointing to the probability of multiple introductions of ASF into Europe. The Lisbon 1957 outbreak can be linked directly to virus circulating in Angola, thereby confirming the belief that this country was the source of the original infection exported from Africa. The results further point to the possibility of reverse virus flow from South America to West Africa in 1982.

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INTRODUCTION

African Swine Fever virus (ASFV) is an icosahedral cytoplasmic deoxyribovirus (ICDV) of the genus *Asfivirus* within the family *Asfarviridae* (Dixon *et al*, 2000). It causes a highly lethal infection in domestic pigs, which cannot be controlled through vaccination. When outbreaks occur, a slaughter-out policy is usually used to eradicate the disease, resulting in severe losses to commercial and subsistence farmers. The economic implications of the ASF are particularly severe to the burgeoning pig industry in sub-Saharan Africa, where the disease is endemic. Virus persistence in Africa is facilitated through one of three possible cycles: (1) an ancient sylvatic cycle in which virus circulates between soft ticks of the *Ornithodoros* complex and wild suids such as warthogs, bush-pigs and the giant forest hog (Plowright *et al*, 1994); (2) a cycle occurring in the endemic region of Malawi which involves domestic pigs and *Ornithodoros* ticks (Haresnape and Mamu, 1986); and (3) a cycle involving domestic pigs in the absence of any known wild suid or invertebrate hosts (Thomson *et al*, 2002). The latter cycle is believed to be present in West Africa as the disease has been present for extended periods: 1982-1989 in Cameroon and 1996-present in numerous West African countries. This despite the lack of evidence for the tick vector and the negative results obtained from serological surveys of wild suids in this region (Leeson, 1952; Ekue and Wilkinson, 1990).

The presence of ASF in Africa was well recognised prior to the introduction of the disease to Europe in 1957. Once exported from Africa, this exotic disease became established in the Iberian peninsula from where it moved to other European countries, to South America and to the Caribbean. Although eradicated from most of the afore-mentioned countries at great expense, the disease still persists in Sardinia. Restriction fragment length polymorphism (RFLP) analysis and nucleotide sequencing indicate that the outbreaks occurring in West Africa, South America, the Caribbean and Europe have a common origin (Wesley and Tuthill, 1984; Blasco *et al*, 1989; Dixon et al, 1990; Bastos *et al*, 2002). Combined results show that at least 24 countries have experienced outbreaks caused by viruses of the ESAC-WA (Europe, South America, Caribbean and West Africa) genotype (Bastos *et al*, 2002). Despite the extensive geographical and temporal distribution of this genotype, both molecular methods (RFLP and sequencing) were unable to discriminate between viruses belonging to this genotype due to genomic homogeneity. The latter essentially means that it is not possible to determine the origin of an outbreak, or the transmission path when a virus belonging to this group is the causative agent. Preliminary evaluation of a central variable region (CVR) encoded within the 9RL open reading frame (ORF) of viruses of the ESAC-WA genotype has identified size heterogeneity in this gene indicative of potential discriminatory powers o this genomic region (Bastos, 2001; Bastos *et al*, 2001). Differentiation between viruses on the basis of size alone is not, however, advised as CVR product sizes overlap between different *p72* genotypes (Bastos, 2001; Bastos *et al*, 2002). Thus CVR characterisation

should only be performed following *p72* genotype determination.

To expand on these preliminary findings and to test the potential of CVR for differentiation between viruses ESAC-WA genotype, isolates causing outbreaks between 1957 and 2000 in West Africa and Europe were selected for genetic characterisation.

MATERIALS AND METHODS

Twenty-seven ASF isolates were selected for CVR-PCR amplification. DNA was extracted using a modification of the silica/guanidium-based method developed by Boom *et al* (1990). The CVR region of the *9-RL* ORF was amplified using flanking CVR primers CVR-FLF and CVR-FLR (Bastos, unpublished). The amplified products were run against a 100 bp ladder (Promega) on ethidium bromide-stained agarose gels. Using the PhotoCaptMw software package (Vilber Lauromat), ten size groupings were identified across all extracted isolates (Table 1), from which twenty-one strains were selected for sequencing, with at least one representative selected from each size group. Nucleotide sequences were determined for both sense and antisense strands with the external PCR primers, by automated DNA cycle sequencing, according to supplier specifications (Applied Biosystems). The nucleotide sequence data generated in this manner were pooled with ten published sequences (Irusta *et al*, 1996). Details of viruses included in this study are summarized in Table 1.

Sequence chromatograms were edited in CHROMAS and exported to DAPSA, version 4.9 (Harley, 2001), where they were translated. Individual amino acid sequences were exported to a word processing program and manually aligned. The diversity of the tetrameric repeats in composition and number required the insertion of gaps (which were negatively weighted) in order to optimise the alignment. Gaps were coded according to the guidelines of Simmons and Ochoterena (2000), following which mean character distances and maximum parsimony (MP) analyses of the coded data set were performed using PAUP*4.0 beta 10 version (Swofford, 2002). UGA 61 and Spencer were specified as outgroups since they are genetically and geographically distinct from viruses of the ESAC-WA *p72* genotype (Bastos *et al*, 2002).

RESULTS AND DISCUSSION

CVR-PCR size determination of the ESAC-WA genotype based on nucleotide sequencing identified ten distinct CVR-PCR size groups (Table 1). Although some ASF strains were identical to each other on the basis of CVR-PCR product size, nucleotide sequencing revealed that they differed in amino acid tetramer composition from others within their group, notably VICT 90/1 and DRC/624/89 (Table 1). These differences were reflected phylogenetically when the ESAC-WA genotype was resolved into 11 discrete CVR-PCR lineages, labelled I-XI (Table 1; Fig. 1).

Table 1. CVR characterisation of ASF viruses of the ESAC-WA genotype, by PCR sizing and nucleotide sequence determination

Isolate	Geographical origin	Year of outbreak	CVR-PCR size	CVR-PCR lineage
#BEN 97/4	Benin	1997	689 bp 1	I
IC 1/96	Ivory Coast	1996	689 bp 1	I
NIG 2/00	Nigeria	2000	689 bp 1	I
NIG 3/00	Nigeria	2000	689 bp 1	I
GHA 1/00	Ghana	2000	689 bp 1	I
#Malta 78	Malta	1978	629 bp 2	II
#VAL 76	Spain	1976	557 bp 3	III
#OURT 88	Portugal	1988	557 bp 3	III
#LIS 60	Portugal	1960	581 bp 4	III
†MADRID	Spain	1975	§557 bp 3	III
†E70	Spain	1970	§557 bp 3	III
†E75	Spain	1975	§557 bp 3	III
†LIS 60	Portugal	1960	§557 bp 3	III
†DRII	Dominican Republic	1979	§521 bp 3	IV
#CAM 89/1	Cameroon	1989	497 bp 5	V
#CAM 1/86	Cameroon	1986	485 bp 6	VI
#CAM 82	Cameroon	1982	485 bp 6	VI
†CAM 82	Cameroon	1982	§485 bp 6	VI
†Brazil 1	Brazil	1978	§485 bp 6	VI
†Brazil 2	Brazil	1978	§485 bp 6	VI
#BEL 85	Belgium	1985	473 bp 7	VII
#HOL 86	Holland	1986	473 bp 7	VII
IC 3/96	Ivory Coast	1996	425 bp 8	VIII
#LIS 57	Portugal	1957	425 bp 8	VIII
#Dakar 59	Dakar	1959	425 bp 8	VIII
#ANG 70	Angola	1970	425 bp 8	VIII
#VICT 90/1	Zimbabwe	1990	425 bp 8	IX
DRC/624/89	DRC	1989	413 bp 9	X
GAM 1/00	Gambia	2000	413 bp 9	VIII
#NUR 1/90	Sardinia	1990	401 bp 10	XI
†UGA 61	Uganda	1961	§353 bp	
†Spencer	O/U	1951	§353 bp	

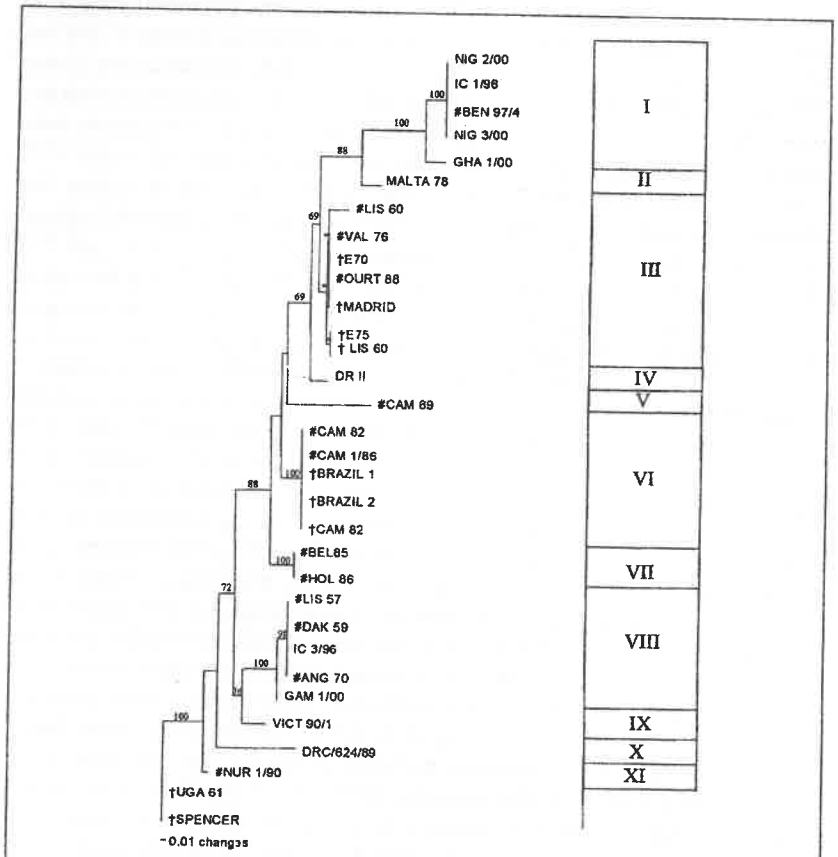
* Indicates samples supplied by the WRL, Pirbright, UK.
# Indicates viruses supplied by the WRL for ASF, Pirbright, UK.
§ Indicates CVR-PCR size estimation based on published data.
† Indicates data obtained from Irusta *et al* (1996).
O/U = Origin uncertain.
Viruses not belonging to the ESAC-WA genotype indicated in bold.

Phylogenetic support for the 11 lineages indicated in Fig. 1 was obtained with both distance and parsimony analyses, confirming that the phylogeny recovered is reliable (Kim, 1993). LIS 60, the ASF strain obtained from the WRL (Pirbright, UK) has two additional tetrameric repeats compared to the sequence of the same virus LIS 60 published by Irusta *et al* (1996). This result suggests that LIS 60

(WRL) was probably more extensively passaged on a monkey cells line than the virus used in the Irusta and co-worker (1996) study, as adaptation to non-porcine cell cultures is known to increase the size of the CVR (Irusta *et al*, 1996). Analysis of the CVR sequences further indicates that the Lisbon/60 virus is unrelated to the Lisbon/57 virus recovered from the initial introduction of ASF into Europe. This supports results obtained in an RFLP study indicating that Lisbon/57 and Lisbon/60 although related, have different *Sal I* restriction enzyme profiles (Blasco *et al*, 1989) and are therefore representative of two separate introductions of ASF into Europe. These two Portuguese strains fall within CVR lineage III and VIII (Table 1, Fig. 1), the former comprising of viruses from Portugal (1960 and 1988), Spain 1970; 1975-1976), whilst the latter incorporates viruses from Portugal (1957), Senegal (1959), Angola (1970) and Côte d'Ivoire (1996). Lineage VIII has been therefore been circulating throughout Europe and Africa (where it is endemic) for almost forty years, and was probably introduced to Portugal in 1957 from Angola via contaminated meat obtained from a flight from Angola and fed to pigs, as has been speculated in literature (Braço Forte, 1970). Lineage III, although in circulation for a shorter period of time (28 years), is also genetically stable in the field. The lack of any African viruses within this lineage makes it impossible to retrospectively determine the source of this introduction. Similarly, the 1978 outbreak in Malta (lineage II), the epidemiologically-linked outbreaks occurring in Belgium and Holland in 1985 and 1986, respectively (lineage VII) and the 1990 outbreak in Sardinia (lineage XI) are unrelated to each other and do not contain viruses of African origin. Together these results point to the possibility of at least four separate introductions of ASF to Europe, of which only one can be directly linked to a specific African country on the basis of anecdotal and molecular evidence.

It is generally accepted that on introduction of ASF to the Iberian peninsula, that the disease spread from this region to the rest of Europe and from there to South America and the Caribbean. Inclusion of viruses from the latter two regions failed to link outbreaks occurring in Brazil and the Dominican Republic with any European strains. However, a direct link between the outbreak occurring in Brazil in 1978 and the 1982 outbreak in Cameroon was obtained, suggestive of reverse flow from the Americas to Africa. However, due to a lack of representation of earlier outbreaks from these regions, the direction of movement remains equivocal. Epidemiological links between these two continents have previously been identified by RFLP analysis (Wesley and Tuthill, 1984). On introduction to Cameroon in the early 1980's, outbreaks occurred in subsequent years up until 1989, and ASF is still reported virtually annually. Analysis of the CVR region shows that the virus introduced in 1982 circulated in Cameroon until 1986, but that the outbreak occurring in 1989 is epidemiologically unrelated.

Figure 1. Neighbor-joining tree depicting CVR sequence relationships of viruses from West Africa and based on mean character distances obtained with the coded CVR dataset



West African CVR lineages are indicated I–XI. * denotes bootstrap values >70%.
† indicates data obtained from Irusta *et al.* (1996).
# indicates isolates supplied by the WRL for ASF, Pirbright, UK.

Six distinct CVR lineages occur within West Africa. Lineage I clearly indicates that the outbreaks affecting Côte d'Ivoire, Bénin, Nigeria and Ghana between 1996 and 2000 are part of the same epizootic, with temporal origins suggesting that the disease spread from Côte d'Ivoire, to Bénin and from there to Nigeria and on to Ghana. As discussed previously, two distinct lineages could be identified in Cameroon, one associated with the outbreaks occurring between 1982 and

1986 and the other with the 1989 outbreak. Lineage VIII is represented by viruses from Senegal, Côte d'Ivoire, Angola and Gambia. This lineage is geographically and temporally the most diverse of the West African lineages and also confirms *p72* genotyping results suggesting that two distinct ASF viruses were circulating simultaneously in Côte d'Ivoire during the 1996 outbreak. The remaining two African lineages, lineage X and XI are represented by a single virus and correspond with *p72* genotyping results (Bastos *et al*, 2002).

Within-lineage variation involving length variation and / or minor sequence variation was observed in lineages I, III and VIII. However, these variations were minor and most likely reflect the high mutational rate within the CVR. Thus, GAM 1/00 is incorporated within lineage VIII on the basis of complete sequence identity with other viruses within this lineage, despite having one tetramer less than the other viruses as this size variation probably arose from either an error in crossover (Watson *et al*, 1987), or through polymerase slippage during replication (Levinson and Gutman, 1987). Similarly, GHA 1/00, although slightly different from other viruses within lineage I on the basis of the sequential order of the tetramers, clearly belongs within this group. Within group III, E75 and LIS 60 (Irusta *et al*, 1996 *sequences*) differed at two amino acid positions from VAL 76, OURT 88, MAD 75 and E70, altering the composition of only one tetramer from CADT to CASM, whilst LIS 60 (WRL isolate) had two additional tetramers.

According to the NJ (Figure 1) and MP tree topology, Malta 78 was closely related to group I. Two theories regarding the origin of this outbreak are proposed. The Malta strain may have been introduced into Europe and its CVR size altered due during field circulation, or Malta 78 is representative of another introduction from West Africa, which is separate to that of LIS 57 and LIS 60.

Comparison of NUR 90/1 with viruses from an unrelated *p72* genotype, namely UGA 61 and Spencer, revealed that although these viruses are similar in size (they only differ by one tetramer), the East African viruses, UGA 61 and Spencer contain a unique 'RAST' tetramer, not observed in any of the ESAC-WA genotype viruses.

In conclusion, CVR analysis of the ESAC-WA genotype reveals that the composition of the repeat region is extremely stable in within CVR lineages, over an extended time period (eg. lineage VIII), despite the hypervariable nature of repetitive DNA. This observation is suggestive of repeat introductions into Europe from Africa, rather than extensive evolutionary changes being introduced during virus cycling on the European continent. However, further investigation of the genetic variability within this genomic region of additional viruses of European and West African origin is required to clarify this. CVR sequence determination has unequivocally shown that this method is suitable for differentiation between geographically, temporally and genetically related viruses, to such a degree that the course and cause of epizootics in West Africa from 1996 to 2000 could be accurately determined.

ACKNOWLEDGEMENTS

We wish to thank the FAO of the UN for financial support. We are also grateful to Geoff Hutchings (WRL, UK), Drs Felicité Beaudjé and Couacy-Hymann (Côte d'Ivoire), Drs Samuel Ofosu and George Opuku-Pare (Ghana), Dr Eunice Forster (Gambia) and Dr. David Olaleye (Nigeria) for providing samples and isolates for this study.

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DISEASE SURVEILLANCE FOR NORTHERN AUSTRALIA

G H PERRY[1] and T D BUICK

SUMMARY

Several disease surveillance programs operate in northern Australia. The Northern Australia Quarantine Strategy (NAQS) is Australia's early warning quarantine defence system that manages the unique quarantine risks associated with the proximity of Australia's northern neighbours, Australia's remote and sparsely populated northern coastline and the presence of pests and diseases of quarantine concern to the north. To enhance the program, NAQS also operates offshore programs in eastern provinces of Indonesia, East Timor and Papua New Guinea. The National Arbovirus Monitoring Program (NAMP) monitors the distribution of economically significant insect-borne viruses of livestock and their vectors, in particular, bluetongue, Akabane and bovine ephemeral fever viruses and provides data for scientifically based risk management strategies. This program enables other countries to develop practical import protocols for Australian cattle. Other country wide animal disease programs of importance in northern Australia include the Tuberculosis Freedom Assurance Program (TFAP) and the National Transmissible Spongiform Encephalopathy Surveillance Program whose main role is to maintain Australia's free country status for bovine tuberculosis and BSE respectively. Consequently, because such programs provide assurance of Australia's animal health status, Australia can now export over 800 000 live cattle and over 4 000 000 live sheep each year, as well as meat and other animal products. The paper briefly describes the key surveillance aspects of these programs.

INTRODUCTION

Disease monitoring and surveillance programs in northern Australia present unique and interesting challenges. Northern Australia is not a defined geographical entity but is a loosely described area of Australia north of the Tropic of Capricorn. Compared with the rest of Australia, northern Australian coastline is remote, sparsely populated and has proximity to northern neighbours where there are pests and diseases which pose quarantine concern. The interior is characterised by remoteness, very large cattle holdings, national parks and wilderness areas. The Australian Quarantine and Inspection Services (AQIS) of Agriculture Fisheries and Forestry Australia (AFFA) manages the Northern Australia Quarantine Strategy (NAQS), Australia's early warning quarantine defence system that manages the special quarantine risks presented by our northern neighbours. Similarly the Queensland Government conducts the Northwatch program in the Cape York Peninsula to increase its ability to resist the threats of exotic pests and diseases posed by its geographical proximity to Papua New Guinea (PNG) and Indonesia.

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In 1977 disease monitoring in northern Australia had uncovered the presence of bluetongue virus in Culicoides. Retrospective serological studies indicated that the bluetongue virus had been in Australia since at least 1958. This led to the establishment of National Arbovirus Monitoring Program (NAMP), an integrated national program managed by Animal Health Australia, to monitor the distribution of economically important insectborne viruses of livestock and their vectors. These include bluetongue, Akabane and bovine ephemeral fever viruses.

According to the OIE International Animal Health Code definitions, Australia is officially free from bovine tuberculosis after a successful 28-year campaign to control and eradicate the disease. The Tuberculosis Freedom Assurance Program (TFAP) was developed to maintain Australia's 'country officially free' status for bovine tuberculosis.

Australian animals are free of transmissible spongiform encephalopathies (TSE's). The OIE International Animal Health Code requires countries that claim to be free of TSE's to have in place a surveillance system for the detection of BSE and scrapie. The National Transmissible Spongiform Encephalopathy Surveillance Program (NTSESP) is an integrated program managed by Animal Health Australia to demonstrate Australia's ongoing freedom from BSE and scrapie, and to provide early detection of those diseases if they occur.

After an eradication campaign that started in 1970, Australia declared its freedom from bovine brucellosis in July 1989 and has remained free.

This paper describes the surveillance and monitoring methods used in these programs.

METHODS

Northern Australia Quarantine Strategy (NAQS)

NAQS activities can be divided into two components, the onshore and the offshore programs. NAQS onshore program operates within a coastal zone extending from Cairns in Queensland including the Torres Strait, through Darwin in the Northern Territory to Broome in Western Australia, with flexibility to include other risk areas in the region, such as farming areas and travel routes. NAQS also has offshore programs in Papua New Guinea (PNG), East Timor and in the eastern Indonesian provinces of East Nusatenggara (West Timor) and Papua (formerly Irian Jaya).

The NAQS program operates by identifying and assessing quarantine risks to Australia from countries to its north and by implementing monitoring, surveillance, public awareness and research strategies to manage these risks. NAQS surveys are designed either to find the pest or disease if present or to gather

disease prevalence information and are implemented according to the area being surveyed.

Using a Scientific Planning Group, NAQS has identified the risks and developed a list of exotic pests and diseases that are targets for NAQS activities. This target list is based on relevant scientific literature, the quarantine status of the pests and diseases and the risk of their entry through northern Australia. The target list is under constant review to take into account changing geographic distribution and emergence of new diseases. Pests and diseases targeted by NAQS are listed in Table 1.

Table 1. Targeted pests and diseases

Target list of diseases and pests	*PNG*	*Irian Jaya (Indonesia)*	*Other Indonesian provinces*	*East Timor*	*Malaysia*
Foot and mouth disease	Never infected	Probably never infected	Free since 1986	Probably never infected	Usually free but experiences incursions from neighbours
Surra (*Trypanosoma evansi*)	Free	Free*	Enzootic	Enzootic	Enzootic
Old World screw-worm fly	Recorded since 1920's	Enzootic	Enzootic	Enzootic	Enzootic
Classical swine fever	Free	Free	Introduced in 1996	Enzootic	Enzootic
Haemorrhagic septicaemia	Free	Free	Enzootic	Enzootic	Enzootic
Japanese encephalitis	Enzootic, with incursions into Australia in 1995 and 1998	Enzootic	Enzootic	Enzootic	Enzootic
Newcastle disease	Free	Enzootic	Enzootic	Enzootic	Enzootic
Infectious bursal disease	Free	Enzootic	Enzootic	Enzootic	Enzootic
Rabies	Free	Free	Enzootic except Bali, NTT and Moluku	Free	Enzootic
Nipah virus	No evidence	No evidence	No evidence	No evidence	Enzootic
Asian honey bee and bee mites	Enzootic	Enzootic	Enzootic	Enzootic	Enzootic

** An inconclusive small cluster of serological positives needs further evaluation*

Other threats believed to be present in countries to Australia's north include exotic strains of bluetongue virus, Aujeszky's disease, porcine reproductive and respiratory syndrome, duck viral enteritis, transmissible gastroenteritis, swine influenza, *Trichinella* sp., *Taenia* solium, avian influenza, duck viral hepatitis, and *Ehrlichia canis*.

Onshore component

NAQS also assesses each geographical region within its on-shore area of operations for factors associated with a higher probability of pest and disease movement. These include proximity to PNG, traditional movement rights of southern PNG inhabitants in the Torres Strait (chain of islands that separate PNG from mainland Australia), history of illegal vessel movements and presence of host species. These Area Risk Assessments classify the NAQS region into five risk zones: very high, high, medium, low and very low. These risk ratings determine the amount and type of activities that are required for each area and survey frequencies are graded accordingly, from once every five years for the very low risk zones to two or more times a year for the very high risk zones.

Surveys are undertaken by ground vehicle or helicopter, and involve domestic, feral and wild animals and bird populations. Feral animals, including pigs, cattle, donkeys, goats and deer are killed and autopsied. Domestic animals are surveyed by observation with collection of information through history taking and clinical and laboratory examination. The surveys in the Torres Strait use Coastwatch helicopters while those in the Northern Peninsular use land vehicles.

Sentinel herds of pigs and cattle are monitored for target diseases including Japanese encephalitis, exotic bluetongue strains, screwworm myiasis and surra. Following the detection of Japanese encephalitis in recent years in the central Torres Strait islands, seronegative pigs are flown to strategic sites in Cape York Peninsula and the Torres Strait at the start of each wet season and are bled fortnightly to monitor for Japanese encephalitis viral activity and are finally tested for Aujeszky's disease, classical swine fever, and porcine reproductive and respiratory syndrome. Birds are trapped and tested for target list avian diseases.

Screwworm fly traps are used to monitor for the presence of adult flies. Bluetongue virus vector trapping is undertaken for NAMP, and opportunistic sampling of feral bee nests is performed to monitor for Asian honeybees and bee mites. There is a small sentinel hive program at Weipa. Quarantine officers stationed on islands in the Torres Strait produce census data of domestic livestock and report the numbers of feral animals where possible.

Public quarantine awareness is an important aspect of the program. People inhabiting or visiting the relevant area are provided with diagnostic pointers for conditions including screwworm myiasis, classical swine fever and foot-and-mouth disease. School demonstrations, Indigenous ranger training, Environment Australia staff training and material for pastoralists, shooters and four-wheel drive clubs are provided. An Indigenous communications strategy was commissioned and a liaison officer will be appointed in the Northern Territory for work in isolated communities. The aim is to get more information from the vast lengths of coastline administered by Indigenous communities.

Information is stored in a database and a summary is provided for NAHIS. No incursion of exotic animal disease was detected on mainland Australia in 2001. Evidence of Japanese encephalitis infection has been found in the central

Torres Strait Islands during each of the last four wet seasons.

Offshore component

Animal health surveys are undertaken at a frequency as determined by the Area Risk Assessment in neighbouring countries to determine changes in distribution of target diseases. The information gathered is used to assess and manage risk to Australia. Veterinarians from the host country and NAQS jointly undertake the surveys, utilising clinical and pathological skills and serology.

Diseases for surveillance for each country are selected from the target list and are based on quarantine significance of a disease to Australia and existing knowledge of its prevalence in the host country. The host country adds its own diseases of concern. The disease target list is flexible and capable of responding to emerging threats. Reports of unusual disease or deaths are investigated where possible and the risk to Australia assessed.

In the countries involved, difficult terrain and conditions influence the sampling locations and numbers, however the probability of finding disease is maximised by biasing the survey to selected areas that have the greatest chance of having or having been exposed to the targeted diseases. Villages and areas are selected on the basis of known risk factors for movement of disease, including host and vector distribution and human habitation and movement. At the village or local level, selection of animals for physical and laboratory examination is based on owner history and movements, husbandry method and individual animal factors, such as age. Autopsies are performed on wild animals or animals exhibiting illness if possible.

NAQS carries out prevalence surveys of domestic animals in Papua and Timor with the required information on livestock numbers and distribution being generally available. NAQS also conducts a monitoring program in PNG using sentinel cattle, pigs and chickens located at several strategic locations throughout the country, particularly in areas adjoining the PNG/Papua border where there is higher risk of potential exposure to the pests and diseases of concern. These animals are generally housed in a village situation and are examined and tested on a quarterly basis for the target diseases.

Detailed information including geographic coordinates and results of testing is recorded in the NAQS database for each sample collected. Results are provided to the host countries and reported by NAQS once cleared by the host government.

Northwatch

Northwatch scientists survey Cape York Peninsula and the Gulf of Carpentaria, as well as large metropolitan areas as far south as Mackay for incursions of plant, animal and insect pests and diseases. The remote areas are nominally divided between Northwatch and the NAQS, but there is overlap and sharing of data. Joint surveys have been successfully carried out with scientists from both agencies working together. Northwatch also contributes to surveillance programs

of other agencies such as NAQS and Queensland Health, by lending its resources and skills.

National Arbovirus Monitoring Program (NAMP)

The National Arbovirus Monitoring Program (NAMP) is an integrated national program jointly funded by industry and governments to monitor the distribution of bluetongue, Akabane and bovine ephemeral fever viruses. While bluetongue viruses occur in Australia, there is no evidence of clinical disease in the field.

NAMP provides data so that Australian and international regulatory agencies can accurately assess the nature and distribution of arboviral infections in this country, thus facilitating trade by promoting confidence in scientifically based risk management strategies. Some countries have developed import protocols for Australian cattle using NAMP information, defining the geographical areas from which cattle may be sourced.

Data is gathered by monitoring cattle located in sentinel herds throughout the country. At sentinel locations, groups of ten young cattle previously unexposed to arboviral infections are blood tested at regular intervals to detect any incidence of infection. Sampling frequency is proportional to the probability of arbovirus activity. Insect traps located near the sentinel animals indicate whether Culicoides vectors are present during the testing period. Sentinel sites are selected to allow plotting of the distribution of infections. Many are positioned in areas along the border between expected infected and uninfected areas, or where infection occurs irregularly.

Expected free areas are monitored to verify their free status. Known infected areas are sampled to assess the seasonal intensity of infection of each virus. At one site in the Northern Territory virus is isolated to detect possible incursions of viruses from overseas.

Tuberculosis Freedom Assurance Program

The Tuberculosis Freedom Assurance Program (TFAP) involves active surveillance through the National Granuloma Submission Program (NGSP) and eradicating any residual cases of tuberculosis detected in the Australian cattle population. Under NGSP any granulomatous lesions detected on postmortem examination of cattle at slaughter are examined for bovine tuberculosis in accredited laboratories. In the year 2001 5 223 lesions were submitted via the NGSP from over seven million adult cattle slaughtered. No bovine tuberculosis was found. In 2000 two granulomas were found to be bovine tuberculosis.

The rapid growth in the export of live cattle from northern Australia has impacted on the NGSP and consequently TFAP. Some 700 000 live cattle are exported from this region and several abattoirs in northern Australia have closed. Because of this, TFAP has been reviewed and active surveillance using tuberculin testing is being extended to target cattle on properties with a previous history of tuberculosis.

National Transmissible Spongiform Encephalopathy Surveillance Program

The primary purpose of the National Transmissible Spongiform Encephalopathy Surveillance Program (NTSESP) is to support trade by maintaining a surveillance system for transmissible spongiform encephalopathies (TSE's) that is consistent with the OIE International Animal Health Code recommendations and which assures all countries importing cattle and sheep commodities that Australia remains free of these diseases.

Accordingly, the components of NTSESP include:

- field investigations of cases of clinical signs of nervous disease where TSE is suspected;
- screening the case histories of all laboratory submissions with a clinical history of nervous disease, to exclude the diagnosis of a TSE; and
- screening the brains of all animals over the age of two years with a clinical history of nervous disease to detect lesions of TSE and establish an alternative diagnosis.

The surveillance program is structured so that there is a 90 per cent probability of finding infection of BSE or scrapie if it occurred in one per cent of the nervous conditions occurring in cattle and sheep in Australia. For the first year of the program, Australia raised the sampling levels to 500 cattle and sheep to provide a 95 per cent probability of finding a 0.5 per cent level of infection.

Bovine Brucellosis

After an eradication campaign that started in 1970, Australia declared itself free of bovine brucellosis in July 1989 and remains free of the disease. This was confirmed by an active surveillance program involving collection of blood samples from all adult female cattle at slaughter until the end of 1993. Since that time, passive surveillance involving serologic tests of cases of abortion and of cattle tested for other reasons, including export testing, has demonstrated ongoing freedom. In 2001 710 cases of abortions were investigated and tested for bovine brucellosis while 41 428 other cattle were tested for various reasons. All were negative.

CONCLUSION

Programs such as those discussed above provide evidence for the continued excellent health status of Australia's animal populations. Consequently Australia is able to export a considerable number of livestock, including over 4 000 000 sheep and 700 000 cattle each year, as well as large volumes of animal products around the world. Disease surveillance is recognised as a major function of government animal health services in Australia. Information on animal health is available from a range of government and non-government sources. To collate the findings of the various surveillance programs, a National Animal Health Information System has been established and provides an overview of animal health in Australia (Website: http://www.aahc.com.au/nahis/index.htm).

ACKNOWLEDGEMENTS

We wish to thank the staff of NAQS, AFFA and Animal Health Australia for providing the information used in this paper. As Australia's offshore programs were mentioned in this report, we also acknowledge the contribution of our counterparts in Indonesia, East Timor and Papua New Guinea to the surveillance and monitoring programs in northern Australia.

SURVEILLANCE AND DISEASE MONITORING IN FREE- RANGING AFRICAN WILDLIFE

R G BENGIS

INTRODUCTION

It is generally recognized that countries that conduct disease surveillance in their wildlife populations are more likely to detect the presence of infectious animal diseases, be they pre-existing or newly established, within their borders (Morner *et al*, 2002). Many indigenous and endemic pathogens frequently cycle 'silently' in their traditional hosts, with little expression of disease. Newly emerging or novel diseases entering an ecosystem are generally present at low incidence or prevalence (Williams *et al*, 2002). These two categories of infections are difficult to detect unless dedicated and targeted surveys are introduced.

Other indigenous pathogens, in order to fulfill their survival, propagation and transmission strategies (e.g. anthrax and rabies), may cause severe disease in wildlife. These diseases generally affect a broad range of species, and are inherently fatal. Clinical outbreaks are frequently driven by climatic factors and host population densities, and are frequently highly visible and dramatic, but usually of short duration and relatively localised in focal or multi-focal clusters.

Alien diseases on the other hand may have devastating effects on indigenous wildlife, and are a major cause for concern because they frequently cycle independently of conventional ecological drivers, and may have wide spatial spread and extended temporal patterns (Bengis, in prep.). Examples are rinderpest and bovine tuberculosis (BTB). Examples of these last two categories of diseases are relatively easy to detect (except BTB) because of their high clinical profiles.

This paper will address disease surveillance and monitoring techniques and strategies that have been found to be functional and practical for use in free-ranging wildlife in National Parks and game reserves.

METHODS

Surveillance, surveys and monitoring

In general, ***disease surveillance*** in free-ranging wildlife is employed to detect the presence of pathogens, as well as emergence or cyclical recurrence of disease outbreaks in wildlife populations.

Disease surveys, however, are generally implemented to evaluate and document the presence, prevalence and spatial distribution of infectious agents or vectors. Many disease surveys are dedicated to specific diseases or vectors known to occur in a specific area. Serological and vector surveys are the two most common techniques utilised. The sampling during these surveys may be random, selective or targeted.

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Disease monitoring, on the other hand, frequently involves repeat surveys and is usually implemented to obtain spatial and temporal information during known disease outbreaks, to document trends and to determine species, age and gender predilection, rate of propagation and other disease determinants (Thrusfield, 1985).

Surveillance techniques and strategies

The responsibility for disease surveillance in domestic animals generally rests with the veterinary regulatory authority of a given country, and the surveillance techniques used may include passive reporting, farm inspections, problem investigations, abattoir surveys, serological surveys and dedicated surveys for specific disease eradication schemes (Thrusfield, 1985). The assistance of private veterinarians, diagnostic laboratories, animal scientists, farmers and breeders association are frequently included.

Wildlife disease surveillance is generally less well structured because free-ranging wildlife are not regularly visited and visualised, frequently do not have owners, and are not easily manipulated for 'hands on' examination or specimen collection (Bengis *et al*, 2002).

For this reason, surveillance techniques for wildlife should be structured to maximize the information gained from the limited availability of captured animals and carcasses that may become available. Examples of various techniques that can be applied are as follows :

- Active investigation of any reports of abnormal clinical signs, mortalities or sustained increase in vulture activity in a given geographical area.
- Diagnostic necropsies on all carcasses that become available on an ad hoc basis. Innovative initiatives, such as collection of road kills or examining hunter kills, can substantially increase the number of carcasses examined.
- Veterinary and veterinary public health inspections at all lethal wildlife population management (culling) operations, as well as livestock slaughter premises in the interface area adjoining conservation areas. Wildlife culls are frequently an excellent opportunity to develop and document inventory lists of pathological processes, micro-pathogens and parasites cycling in a specific population.
- Active veterinary participation in protected area management, with emphasis on training of technicians, rangers and field biologists with regards to specific diseases and their clinical signs, surveillance and sampling techniques, data collection, reporting and decision making.
- Veterinary examination of all animals captured for any reason at all, including translocation, clinical assistance, fitting radio transmitters, or removal of problem animals.
- Veterinary supervision at all wild animal holding facilities and game sales.
- Dedicated or targeted surveys are also an excellent, though expensive, sur-

veillance technique. The value of serum and tissue banks for retrospective studies cannot be over-emphasized.

In all the above-mentioned 'hands on' situations, sample collection, including body fluids, tissues, excretions and parasites should be maximized.

Additional indirect techniques for disease surveillance may include :

- Vector trapping for distribution studies (e.g. Tsetse flies, mosquitoes, *Culicoides*, ixodid and argasid ticks, etc.) or pathogen isolation and xenodiagnosis.
- Rodent trapping for serological surveys (arbor and cardio-viruses) or disease agent isolations.

The identification and confirmation of the aetiological cause of morbidity or mortality in wildlife disease outbreaks is paramount. Once the aetiological agent has been positively identified, then monitoring actions should follow to ascertain trends, and evaluate the importance or impact of the disease at the population level. The potential impact of the specific disease on regional domestic livestock, its zoonotic potential and trade sensitivity are also extremely important considerations. Only after considering all these aspects should multi-disciplinary decisions be made with regards to appropriate disease management, where necessary.

Surveillance and monitoring in Kruger and other national parks: disease patterns

Anthrax

Although anthrax was first diagnosed in the Kruger National Park (KNP) in 1959, there is irrefutable evidence that *Bacillus anthracis* has been present in this ecosystem for many centuries (De Vos, 1998). It is thus considered to be an indigenous disease, and certain endemic foci are the source of infection that initiate the recurrent epidemics that occur cyclically, approximately every ten years. In Etosha National Park anthrax follows a more endemic pattern, with cases occurring annually (Lindeque and Turnbull, 1994).

Epidemics have a typical zonal propagating pattern, and usually occur during the driest period of the year. They are short lived and are dramatically terminated or interrupted by the onset of the rainy season. Anthrax generally moves through an area in a wave-like front, with a high incidence of cases at the frontal surface, and only sporadic cases occurring behind it. In Kruger anthrax is a multi-species disease, but kudu and buffalo form the bulk (80 per cent) of the victims (De Vos and Bryden, 1996).

Anthrax surveillance is mainly executed by trained field staff, including rangers, game guards, biologists and veterinary staff. The start of an outbreak is frequently characterised by a sustained increase in vulture activity in a given area, and finding untouched carcasses in good body condition. Field staff are issued with a blood smear collection kit, wrapped in a small data sheet. Peripheral blood smears are taken from all suspect carcasses, the data sheets are completed and the samples are then sent through to the State Veterinary Investigation Centre at Skukuza for staining and microscopic examination, and /or culture if necessary. Once an outbreak has been detected, then activities shift up a gear into active monitoring mode, involving moderate-scale deployment of staff, vehicles, a mobile laboratory and a helicopter. A central command centre is established in the nearest rest camp, and data collected is stored and mapped on a daily basis to identify spatio / temporal trends (De Vos and Bryden, 1995). Circling and descending vultures are one of the most important indicators for pin-pointing carcasses. The position of carcasses spotted from the helicopter are marked with toilet paper streamers in the top of the nearest tree, and frequently a paper trail (in the tops of trees) is created to the nearest access road, so that ground vehicles can locate and deal with the carcass. GPS systems will also be employed in future outbreaks. Containment and control exercises include incineration or burying of carcasses, disinfection of man-made drinking troughs, strategic veld burning, and vaccination of endangered species and important disease amplifiers.

Foot and mouth disease

The endemic cycle of foot and mouth disease (FMD) is maintained in buffalo herds with virus cycling between adult carriers and the annual calf cohort component (Thomson *et al*, 1992). Buffalo are seasonal breeders, with most calves being born between December and March. Buffalo calves receive colostral antibodies against FMD from their dams, and this passive immunity wanes at between five and nine months of age. Thus most juvenile buffalo become susceptible to infection during the dry season of mid-winter and early spring, when many species are congregating at the remaining permanent water points. During primary infection, buffalo calves shed large amounts of virus, and the infection (usually sub-clinical) rapidly spreads to the other buffalo calves in the herd, and may spill over into other sympatric cloven-hoofed species, resulting in an epidemic cycle. These epidemic cycles are the periods of greatest risk for the spread of infection over the KNP boundaries into the livestock of adjoining communities.

In the KNP impala are the most numerous of the wild cloven-hoofed ungulates. They are highly susceptible to FMD infection and develop clinical disease when infected. To detect FMD epidemic outbreaks impala are targeted as a disease indicator species, and targeted surveillance of impala herds by veterinary field staff is an ongoing activity (Bengis *et al*,1994). Clinical signs of FMD in impala include pilo-erection (febrile response), 'walking on eggs', weight shifting from one limb to another, overt lameness, lagging behind the herd and lying down. Animals with clinical signs are sampled non-lethally or lethally, to obtain blood and tissue samples for virus isolation and serology. It is important to 'fingerprint' the virus causing each outbreak, in order to evaluate the coverage by vaccine strains used in cattle in the adjoining buffer zone. During epidemic outbreaks clinical disease is frequently also diagnosed in kudu, and less frequently in giraffe, bushbuck, nyala and warthog.

Bovine tuberculosis

Bovine tuberculosis (BTB) is an alien disease that probably entered the African continent with cattle brought from Europe by colonial settlers (Henning, 1956). It is a disease with a wide host spectrum, and has opportunistically entered several free-ranging populations of buffalo (Guildbride *et al*, 1963, Woodford, 1982; Bengis *et al*, 1996, De Vos *et al*, 2001), kudu (Thorburn and Thomas, 1940, Keet *et al*, 2001) and lechwe (Gallagher *et al*, 1972). These species all appear to be efficient maintenance hosts, with aerosol transmission predominating. 'Spill-over' of infection also occurs into predators and scavengers that ingest infected material, and infection frequently involves the mesenteric lymph nodes, with secondary haemotogenous spread to distal sites, including bone, lungs, spleen, kidney and serosal surfaces (Keet *et al*, 1996; Keet *et al*, 2000).

BTB is a slow progressive disease with a long sub-clinical phase, lasting months to years. Only animals with disseminated or advanced disease show any clinical signs, which may include coughing, emaciation, staring hair-coats and lameness.

Kudu are an exception, frequently developing overt swellings of one or more of the head nodes, at a relatively early stage. The parotid lymph nodes, in particular, tend to enlarge massively due to abscess formation, and fistulous tracts draining muco-purulent material are commonly seen below the ears. In other species however, BTB surveillance relies heavily on targeted surveys, employing lethal sampling and detailed diagnostic necropsies, *or* non-lethal sampling with ante mortem testing using the intradermal tuberculin tests, or where available, certain blood-based tests. There are unfortunately no sensitive or specific diagnostic tests currently available for pachyderms.

Rinderpest

Although rinderpest has not reappeared in southern Africa, since the devastating pandemic at the turn of the last century (Rossiter, 1994), several outbreaks have been documented in eastern Africa over the past decade (Kock *et al* , 1999), with the emergence of a lineage 2 strain of virus (Barret *et al*, 1998) which is mild in cattle, wildebeest and warthog, but highly pathogenic in buffalo and tragelaphids. Surveillance for rinderpest now frequently relies on the detection of clinical disease and mortality in wildlife, as well as dedicated serological surveys in the various free-ranging and unvaccinated wildlife populations. These surveys not only detect that the populations have been exposed to rinderpest, but age stratification may give valuable temporal information.

Rabies

On the African continent, rabies has been diagnosed in 33 carnivorous species and 23 herbivorous species, with a regional variation of dominant role-players (Swanepoel, 1994). In spite of this, by far the largest number of rabies cases reported in the developing world, occur in domestic dogs, with 'spill-over' into domestic livestock, humans, and wildlife. Surveillance for rabies involves the sampling of individuals of any species that display inappropriate or abnormal behaviour. The abnormal behaviour may include extreme aggression, dumbness, tameness, paralysis, hypersexuality and excessive vocalisation. Salivation and an inability to drink or swallow may be seen.

CONCLUSION

This paper attempts to illustrate the multi-faceted approaches that are required to establish a meaningful disease surveillance system for free-ranging wildlife populations. It is stressed that information gained at all 'hands on' activities involving wildlife should be maximized, diagnostic necropsies should be detailed and intensive, and data collection, storing and management must be efficient. The value of serum and tissue banks for retrospective studies and analysis is also emphasized.

Different patterns of disease require their own specific surveillance techniques, and many wildlife disease events are climate or population density driven. Understanding the epidemiology of most wildlife diseases will result in more efficient surveillance, and in turn, this improved surveillance and monitoring will result in better understanding of spatio / temporal events, and disease patterns.

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ASSSESSMENT OF SURVEILLANCE AND CONTROL MEASURES FOR CONTAGIOUS BOVINE PLEUROPNEUMONIA IN GHANA

P K TURKSON

SUMMARY

Contagious Bovine Pleuropneumonia is endemic in West Africa. In Ghana nomadic cattle from neighbouring countries have been suspected as sources for outbreaks. There is very little published information on CBPP in Ghana.

The local production of CBPP vaccine in 1936 resulted in fewer outbreaks in the north in the 1940s and 1950s. However, in the 1970s and 1980s, there was a resurgence of the disease in Ghana attributed to the economic difficulties that made the purchase of vaccine and organization of vaccination campaigns difficult.

CBPP outbreaks from 1991-2001 were reported in six out of the ten regions of Ghana – Volta, Greater Accra, Brong Ahafo, Ashanti, Northern and Eastern regions. No reports were made from Central, Western, Upper East and Upper West Regions, the last two being major cattle producing areas and directly bordering Burkina Faso. It is doubtful that these four regions are free of CBPP. The suspicion is that CBPP cases are being missed or go unreported. It may be necessary to institute a vigorous epidemio-surveillance system involving close monitoring by field staff and in the abattoirs to ascertain the status of the disease here. For the period 1991-96, few (less than five) outbreaks per year were reported. However, in 1997 43 outbreaks were reported. From 1997-2000, the average number of outbreaks reported was 32 (minimum 16; maximum 48). However, cattle losses associated with these were low.

The only surveillance system for CBPP in Ghana at the moment is field surveillance and reporting. Slaughterhouse surveillance and trace back is hardly used. Control measures have mainly been movement control and quarantine. However, the enforcement of these, especially for nomadic cattle, suspected to be the sources of most outbreaks, has been difficult. Vaccination coverages have consistently been low being less than ten per cent per year from 1986-2000. This has been attributed to shortage of vaccines and lack of mobility.

The control options available in Ghana are movement control and vaccination of nomadic cattle where possible and practicable, and strengthening of epidemio-surveillance and trace back procedures through meat inspection, training of farmers and field staff to recognize the disease and improvement in laboratory capacities for CBPP diagnosis. It is recommended that these be done in tandem with control measures in the neighbouring countries on a regional basis because of the ease of transboundary movement of cattle in the West African sub-region.

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INTRODUCTION

Contagious Bovine Pleuropneumonia has been identified as one of the major trans-boundary diseases in West Africa (Anosa, 1995). The disease is said to be endemic in West Africa due to

- difficulties in controlling animal movement especially nomadism in the Sahelian countries;
- high concentration of cattle at market places, on pastures and around wells and other sources of water;
- inadequate information on the epidemiological parameters of CBPP;
- some vaccination campaign-related problems such as inefficient planning and organization, inadequate or non-existent cold-chain, lack of control over quality of vaccines used, and low immunity coverage;
- short post-vaccinal immunity;
- difficulties in early detection and diagnosis of the disease since well-equipped laboratories and personnel are inadequate;
- difficulties in implementing sanitary control measures such as quarantine, slaughtering etc;
- reluctance of some farmers to vaccinate their animals (especially taurine breeds) with T1-44 vaccine because of post vaccination reactions;
- laxity in implementation of animal disease legislation related to CBPP control;
- attempts to treat sick animals with antibiotics;
- lack of coordination in CBPP control across countries despite the importance of cross-border cattle movements; and
- armed conflict within and across some countries that do not allow any action to be taken against CBPP (Kane, 2000).

Seifert (1996) noted that an increase in trans-boundary migration of cattle during the peak of the dry season in West Africa as nomads entered agricultural areas in the south with their zebu cattle lead to severe outbreaks of CBPP in the local animals. He stated that a major problem for control is the awareness of nomads or pastoralists that the disease runs a relatively mild course in their animals and may therefore hide sick animals till such animals recover, therefore making any eradication by slaughter scheme difficult or untenable in traditional African livestock production systems. CBPP, he observed, can only be prevented efficiently by regular annual vaccination of all susceptible animals. Provost (1981) postulated that the disease would disappear if 100 per cent of a cattle population older than six months were vaccinated continuously for three to five years. Prior to this, all clinically and chronically infected animals will have to be removed under this scheme.

CBPP affects production and productivity. It has both direct and indirect economic losses seen as mortality, loss of weight, reduced working ability, reduced

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The control options available in Ghana are movement control and vaccination of nomadic cattle where possible and practicable, and strengthening of epidemio-surveillance and trace back procedures through meat inspection, training of farmers and field staff to recognize the disease and improvement in laboratory capacities for CBPP diagnosis. It is recommended that these be done in tandem with control measures in the neighbouring countries on a regional basis because of the ease of transboundary movement of cattle in the West African sub-region.

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CBPP affects production and productivity. It has both direct and indirect economic losses seen as mortality, loss of weight, reduced working ability, reduced

fertility, reduced growth rate, costs of vaccination campaigns, loss of trade due to quarantine and restriction of cattle trade resulting from closure of cattle markets (Masiga and Domenech, 1995).

CBPP is a notifiable or scheduled disease in Ghana. There has been some sort of generic surveillance and control procedures for years for all scheduled diseases. The aim of this paper is to assess whether these surveillance and control measures are appropriate and adequate for CBPP in Ghana, making recommendations where necessary.

METHOD

The method used was the examination of monthly veterinary returns and annual reports of Veterinary Services Directorate from 1974 to 2001 (where available).

Also, there were field visits to observe at first hand procedures in place for surveillance and control, during which veterinary personnel in the field, at the laboratories and at the Directorate were interviewed informally.

Animal population figures for years after 1996 used in this study were all estimates, since the last credible national livestock census was done in 1996.

RESULTS AND DISCUSSION

CBPP outbreaks in Ghana

Nomadic and trade cattle from Burkina Faso and beyond have been suspected of and blamed for outbreaks of CBPP in Ghana. Indeed, cattle farmers and butchers interviewed in 2001 were unanimous in associating CBPP outbreaks with the zebus and other cattle managed and traded by nomadics (Turkson, 2001). ILCA (1979) reported that CBPP is one of two major contagious diseases in Burkina Faso spread through trade and transhumance. The indication of the importance of foreign sources of trade cattle in Ghana is given by the high proportion of such animals in the number of animals slaughtered. For example, from January to September 2000, out of 532 570 cattle slaughtered at the Kumasi Abattoir, 56 per cent came from Burkina Faso (48 per cent) and Togo (eight per cent) (Turkson, 2001).

The disease is said to be endemic in Ghana now and had previously been reported from all the regions of the country. Knichel and Gyening (1989) reported that CBPP was spreading from known foci to previously free areas and it was difficult maintaining effective control measures due to shortage of vaccines and lack of mobility.

Table 1 gives the reported numbers of outbreaks and animal losses associated with CBPP from 1978 to 2001. Over the past ten years (1991-2001) the reported number of outbreaks from 1991-96 were equal to or less than five a year. However, between 1996 and 1997, there was a nine-fold increase in number of reported CBPP outbreaks from five in 1996 to 43 in 1997. This was sustained from

1997 to 2000, where the yearly average number of reported CBPP outbreaks was 32 (minimum 16; maximum 48). These increases could be real or could be due to increased vigilance, awareness or motivation to report outbreaks or improved surveillance. It is worthwhile noting that although there was a significant increase in the number of reported CBPP outbreaks comparing the 1991-96 and 1997-2000 periods, the total cattle losses for the latter period were few, yearly average being 18 (ranging from four to 45). A similar trend of a high number of reported outbreaks but low number of cattle losses may continue in 2001 as shown by the data from January to May (Table 1).

Table 1. Reported CBPP outbreaks and losses in Ghana from 1978-2001

Year	*Number of outbreaks*	*Total loss*
1978	43	557
1979	72	619
1980	31	448
1981	12	42
1982	8	195
1983	18	381
1984	9	46
1985	11	28
1986	4	6
1987	2	2
1988	6	15
1989	4	55
1990	14	44
1991	2	5
1992	0	0
1993	2	N.A
1994	3	1
1995	1	0
1996	5	6
1997	43	11
1998	48	45
1999	16	4
2000	22	12
2001 (Jan-May)	16	7

In the past ten years (1991-2001), CBPP outbreaks have been reported from only six out of the ten regions in Ghana. These are Volta, Northern, Greater Accra, Brong-Ahafo, Ashanti and Eastern regions. No recent reports have come from Central (last reported before 1974), Upper East (last reported 1981), Western (last reported 1985) and Upper West (last reported 1979). Whether these four regions had definitely had no outbreaks or outbreaks had not been reported could not be ascertained. One reason for absence of reports could be the relatively ineffective surveillance systems in these regions for reporting of CBPP.

Surveillance strategies in Ghana

Assessment of CBPP Surveillance set-up

There has been no well-laid out programme for epidemio-surveillance of CBPP *per se*. There are no logistics – vehicles, motorcycles, funds and the necessary support for CBPP surveillance at the moment. The Epidemiology Unit has a computer with TAD-info and GIS software. Two of the three epidemiologists in the Veterinary Services Directorate are experts in TAD-info. Epidemiological clusters covering the whole country have been planned but are yet to be functional. Few GPS are available especially in the northern sector for global position identifications during outbreaks. Interviews with field staff revealed general low morale or motivation, due mainly to poor pay and working conditions, lack of the necessary logistical support for field and laboratory work. Two specific surveillance procedures are discussed below.

Field surveillance and reporting

This is, perhaps, the only surveillance system in place for CBPP in Ghana at the moment. Outbreaks are to be mandatorily reported by field staff since CBPP is a scheduled disease. However, expert opinion seems to suggest serious underreporting of CBPP cases as a number of outbreaks may go unreported.

Turkson (2001) observed that in almost all reported CBPP outbreaks in Ghana, there were no laboratory confirmations. In effect, these could be classified as suspected outbreaks. The absence of laboratory confirmation raises concerns as to whether some of the reported outbreaks are really CBPP outbreaks, since certain diseases, for example, hemorrhagic septicemia, which is a differential diagnosis for CBPP, occurs in Ghana. Every effort has, therefore, to be made to establish an effective field-laboratory linkage for confirmation of all suspected cases. Field and slaughterhouse staff need to be trained to routinely submit samples to the laboratory. The laboratory on its part will have to be committed to providing feedback and having as short a turn-around time as possible. This should not be more than two weeks from date of submission of sample.

Slaughterhouse surveillance and traceback

In most urban areas, slaughterhouses/slabs or some forms of structures to facilitate meat inspection are available. The design and maintenance, however, may be suspect, as a number of them do not have any running water or proper disposal/sanitation facilities and are poorly equipped. Who should provide the facility may sometimes be contentious, as some local governments do not want to build these. District Assemblies will need to be encouraged to build and maintain slaughter facilities as significant portions of the revenue for slaughter go to them.

Slaughterhouse surveillance for CBPP is very limited. From January to June 2001, no cases of CBPP were reported at Tema and Accra Abattoirs at meat inspection (Turkson, 2001). Kumasi, Kintampo and Tamale slaughterhouses had 13, four and five cases respectively between January and May 2000. Juapong slaughter slab had found seven cases between March and May 10, 2001. Visits to and perusal of veterinary returns revealed no reports of CBPP cases in Gushiegu

slab (largest and most active cattle market in the northern part of Ghana), Bolgatanga and Bawku slaughterhouses (over past three years), and Navrongo (since 1985). These are slaughterhouses situated in the major cattle producing areas of Ghana and therefore may give a good indication of the spread of CBPP.

However, the slaughterhouse diagnosis data may be deceptive. This is because lung conditions are routinely grouped together for reporting as pneumonia without any differentiation in most reports. Therefore in early stages of the diseases or where the lesions are not prominent, CBPP may be missed. This contributes to the under-reporting. Furthermore, there is widespread slaughter of animals outside approved slaughter facilities, resulting in the non-reporting of conditions seen at slaughter.

Also, there is no system in place for compulsory trace-back of suspected cases. As observed by Turkson (2001), the major emphasis during meat inspection training and practice is to make immediate judgement calls as to the wholesomeness of meat. Tracing back conditions found at post mortem, or following through with laboratory confirmation tend to be secondary and of least importance. There is therefore the need to redirect meat inspection training to include skills to capture the epidemiological potential in the data generated from meat inspection.

Control strategies

Three methods have been used in the management of CBPP outbreaks in Ghana (Oppong, 1999). These are quarantine, isolation and slaughter of affected animals. The successful production of CBPP vaccine locally at Pong-Tamale in 1936, which allowed introduction of mass vaccination campaigns, is credited with the few outbreaks of CBPP in the northern regions in the 1940s and 1950s. From then the disease was controlled till its resurgence in the 1970s and 1980s. The strategies in place now are vaccination, movement control and quarantine

Table 2. CBPP vaccination coverages in Ghana from 1986 – 2001

Year	*Cattle population*	*# vaccinated*	*% coverage*
1986	1 134 870	104586	9.2
1987	1 169 837	14956	1.3
1988	1 143 812	88714	7.8
1989	1 136 421	21218	1.9
1990	1 144 787	76219	6.7
1991	1 194 633	4202	0.4
1992	1 159 431	7942	0.7
1993	1 168 640	21259	1.8
1994	1 216 677	11291	0.9
1995	1 122 730	5800	0.5
1996	1 247 861	6593	0.5
1997	1 267 826	1846	0.1
1998	1 270 000	0	0.0
1999	1 290 000	8270	0.6
2000	1 300 000	4725	0.4
2001 (Jan-May)	1 315 951	2434	0.2

and recommended slaughter of affected animals.

Vaccination

Table 2 shows vaccination coverages from 1986-2001. It is clear from these that vaccination coverage never went beyond ten per cent in any year in the period under review. Knichel and Gyening (1989) attributed the low vaccination figures obtained from 1983-85 to shortage of vaccines and lack of mobility. These may be true for most of the other years. There is evidence that vaccination coverage proportions rise significantly when staff are motivated financially and morally and vehicles and fuel are made available.

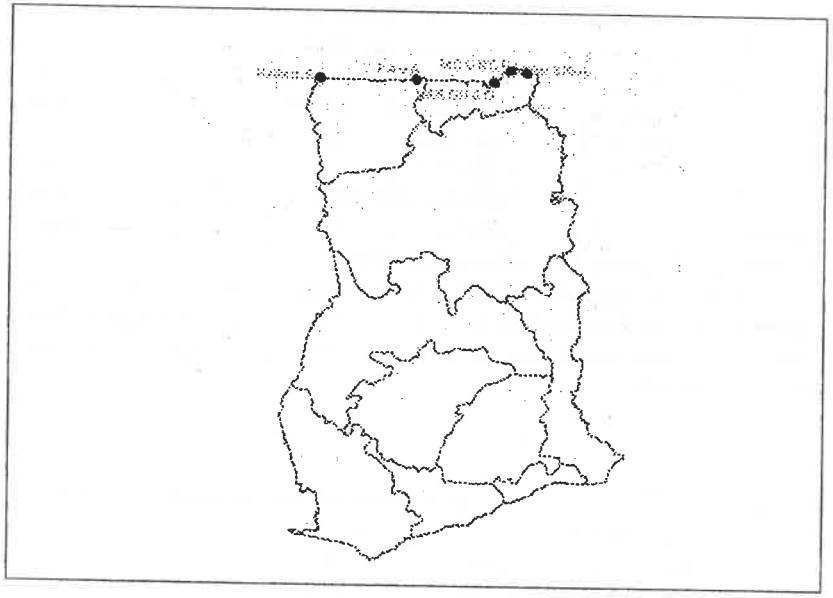
If Ghana is to be on the pathway of eradication to be declared free from CBPP, such low vaccination coverage will not provide the needed impact to make eradication possible.

Movement control, quarantine and slaughter

Movement control and quarantine are the pillars of CBPP control in Ghana. On suspicion of an outbreak, quarantine is imposed in the affected area and movements to and out of the affected and neighbouring areas are restricted. Cattle owners are encouraged to slaughter affected animals. However, the strict enforcement of movement restrictions is sometimes difficult as the law enforcement agencies are not aware of or involved in the implementation of such orders. Masiga and Domenech (1995) noted that quarantine is effective if it is imposed for a minimum of three months and is combined with the slaughter of all positive animals. The enforcement of such a strategy in Ghana will be fraught with frustrations. This is because farmers are reluctant to kill CBPP-suspected animals until they see overt signs of the disease by which time infection may have spread to other in-contact animals. ILRAD (1990) noted that in many parts of Africa it is difficult and almost impossible to impose quarantines or to restrict movement of livestock, and the uncontrolled movement helps spread diseases. Furthermore, slaughter policies are not feasible since the destruction of animals without compensation would also destroy the livelihoods of peasant farmers, as governments cannot afford to pay compensation.

There are quarantine stations in the Upper East and Upper West Regions to control transboundary movement of livestock from neighbouring countries. They are at Mognori, Pusiga, Kasongo, Paga and Hamale. Their locations are shown Figure 1. There is one proposed for Feo, in Bongo District. Generally, the stations are barely functional. At Paga, Mognori and Pusiga, the structures are in advanced stages of disrepair with only the loading ramps being used. The stations at Kasongo and Hamale are relatively better, although not good enough. Hardly any proper quarantine procedures are enforced. All the stations are not fenced allowing mixing of local animals with those supposed to be in quarantine. Since kraals are not available at the stations or if available are unsafe, after reporting the arrival of animals for inspection, owners send these animals to private kraals to rest for some period (ten to 14 days) before being sent south.

Figure 1. Map of Ghana showing quarantine stations



During the period in the kraals these animals are grazed and watered in the communities mixing with the local animals, thereby defeating the very essence of quarantine. Quarantine is critical, since there is a strong contention that CBPP is maintained in Ghana through trade and nomadic cattle. Considering the importance and effectiveness of quarantine and border control in preventing introduction or re-introduction of infection, the repair of these stations and enforcement of quarantine procedures is very critical.

Options available for CBPP control in Ghana

According to a Veterinary Services Directorate, a provisional national eradication strategy for CBPP had been prepared. However, there has been low vaccination coverage over the years. The major problems identified by VSD as militating against effective CBPP control and eradication are:

1. Difficulty in controlling cattle movement into and within the country especially across the northern borders.
2. Complications of applying quarantine and slaughter policies.
3. Lack of data on economic impact of the disease.
4. Absence of a pen-side test for diagnosis.
5. Relatively short period of post-vaccinal immunity .
6. Inadequate funds to implement CBPP programmes.

The major outbreaks or infections in Ghana are suspected to have animals from Burkina Faso and the Sahelian countries as the source. The strategy will therefore be to protect local animals from infections from such sources and also

to contain outbreaks before they spread. The steps that may be used include:

- Control of movement, especially the nomadic cattle;
- Vaccination of nomadic cattle, where possible and practical;
- Strengthening of epidemiosurveillance and trace back procedures through meat inspection, training of farmers and field staff to recognize the disease and improvement in laboratory capacities for CBPP diagnosis.

The most efficient means of CBPP detection in Ghana will be through effective meat inspection procedures in slaughterhouses, followed by laboratory confirmation of suspected cases and trace back of confirmed cases to the origin to help in understanding the epidemiology of the outbreak.

It is clear that controlling CBPP in Ghana alone without concurrent and sustained control in Burkina Faso and the other neighbouring countries will be of no use. This calls for the epidemiosurveillance and control of CBPP to be done on a regional basis.

CONCLUSION

The challenges to surveillance and control of CBPP in Ghana include:

1. Illegal cattle movement.
2. Dominance of imported animals in the cattle trade.
3. Broken down quarantine facilities and procedures.
4. Absence of field-laboratory linkage for laboratory confirmation of suspected field cases or outbreaks.
5. Absence of or limited awareness of or inadequate publicity of declared policies on vaccination (whether to vaccinate or not to vaccinate) and no treatment of CBPP cases.
6. Absence of logistical support in the form of vehicles, funds, and equipment for epidemiosurveillance and vaccination campaigns.
7. Illegal slaughter of animals outside slaughter slabs/ houses and absence of slabs in a number of towns and villages for inspections to be done and serve as first lines of detection of CBPP cases and outbreaks.
8. Absence of economic impact assessment data to help justify CBPP control strategies.

To be able to take care of these challenges, the following recommendations are being made:

1. All suspected CBPP cases should, mandatorily, be confirmed by laboratories.
2. Enforcing existing legislation on meat inspection and local byelaws should stem illegal slaughter outside approved facilities.
3. The quarantine stations need to be rehabilitated and equipped to be functional and quarantine procedures enforced.
4. Active surveillance should be instituted to detect new cases and to confirm the absence of the diseases in areas that have not reported any outbreak for a while since their last vaccinations.

5. Provision of data on economic importance of CBPP in Ghana to justify control measures.

It is envisaged that these will help in stemming outbreaks or spread of CBPP in Ghana.

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SALMONELLA SURVEILLANCE IN DAIRY CATTLE IN ENGLAND AND WALES: A COMPARISON OF RESULTS FROM A SURVEY OF RANDOMLY SELECTED DAIRY HERDS WITH RESULTS FROM STATUTORY REPORTING OF LABORATORY ISOLATES FROM CATTLE WITH CLINICAL DISEASE

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SUMMARY

Current passive surveillance of *Salmonella* in cattle in Great Britain (GB), which includes England, Wales and Scotland, is likely to underestimate true levels of infection and be influenced by submission biases. Over 80 per cent of *Salmonella* incidents reported under Statutory surveillance are the result of diagnostic investigations of cattle with clinical disease. Thus the level of under-reporting, particularly with respect to those *Salmonella* serotypes which rarely cause clinical disease in cattle (e.g. *S.* Agama and *S.* Agona), could potentially be high. The lack of available denominator data also means that it is impossible to estimate the true prevalence and incidence of *Salmonella* within the British cattle population from these data. The Veterinary Laboratories Agency (VLA), United Kingdom, conducted a randomised cohort study from October 1999 to January 2001 to estimate the farm level prevalence and incidence of *Salmonella* on dairy farms in England and Wales. Here the results from this study are compared with the passive surveillance data recorded for diagnostic submissions from cattle in 2000 through Statutory reporting (excluding research data). The regional and seasonal distributions of these reported *Salmonella* incidents were also compared with the cohort study data.

In the cohort study, 449 dairy farms were initially sampled, using environmental sampling, and 272 of these farms were then sampled on three further occasions. The farm-specific prevalence of *Salmonella* (all serotypes) ranged from 12.1 per cent (95 per cent confidence interval (CI): 8.2 – 16.0) to 24.7 per cent (95 per cent CI:19.4 – 30.1) at each visit and the farm incidence rate of *Salmonella* (all serotypes) was 0.43 (95 per cent CI: 0.34 – 0.54) cases per farm-years at risk. The number of *Salmonella*-positive farms was higher than expected from Statutory reporting which identified 889 *Salmonella* incidents (excluding research data) from all cattle in 2000.

All the serotypes isolated in the cohort study were also detected by Statutory reporting during the same period and there were no Statutory *Salmonella* reports

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from any of the farms which were negative throughout the study. Of the 159 farms that were positive at one or more visits during the cohort study, only six reported *Salmonella* incidents between study visits indicating a high level of under-reporting. Reasons for the observed differences between the cohort study and passive surveillance data, for example variation in the proportions of incidents by region and by serotype, are discussed here.

INTRODUCTION

Salmonellosis is an internationally important zoonotic disease (Gomez and others, 1997). The economic cost of *Salmonella* infections exceeds millions of pounds each year as a result of human salmonellosis and increased morbidity and mortality in cattle. Human salmonellosis cases have been attributed to the consumption of contaminated dairy products and direct or indirect contact with infected dairy herds (Wall and others, 1994) and also with consumption of infected meat or products derived from infected animals or cross-contamination.

Cattle are considered an important reservoir of *Salmonella* for man (Calvert and others, 1998). The occurrence of salmonellas in the general cattle population is important from a public health perspective, but is not measured by current surveillance activities. The introduction of the Zoonoses Order in the UK in 1975 (reviewed and updated in 1989 – HMSO 1989) meant that isolation of *Salmonella* from food animals became reportable (Wray and Davies, 2000). Reports of *Salmonella* isolations from cattle (and other livestock) occur primarily through laboratory diagnoses for diseased cattle (VLA, 2000; VLA, 2001) with few data available for healthy cattle populations. This passive surveillance system provides a means of estimating the level of salmonellosis in livestock within the UK and investigating trends in reported incidents[1] over time.

A large-scale cohort study was conducted by the Veterinary Laboratories Agency (VLA) from October 1999 to January 2001 to examine the epidemiology of *Salmonella* on dairy farms in England and Wales. The aims of the study were to estimate the dairy farm prevalence and incidence of different *Salmonella* serotypes in England and Wales, and identify associated risk factors for *Salmonella* in these herds using a standardised environmental sampling protocol. This paper presents the prevalence and incidence estimates obtained from the cohort study and compares these results with reports from the passive surveillance system in 2000.

[1] An incident comprises the first isolation and all subsequent isolations of the same serotype and phage/definitive type combination of a particular *Salmonella*, from an animal, group of animals, or their environment, on a single premises, within a 30 day period (*VLA 2001*).

MATERIALS AND METHODS

Cohort study

Study Population and Farm Selection Criteria

Of 1 224 dairy farms associated with the five milk companies (which represented 63 per cent of dairy herds in England and Wales in 1999), 449 farms were enrolled. 272 farms were selected for follow-up visits. Sample sizes were sufficient to estimate a prevalence of 30 per cent (± five per cent) with 95 per cent confidence.

Sample collection and microbiological testing

Table 1 shows the number of farms sampled at each visit. Environmental samples were collected using a standardised environmental sampling protocol. 20 pooled samples (faecal and slurry) were taken from seven sites including cattle housing, collecting yard, parlour entrance and slurry. All samples (25g per sample) were cultured for *Salmonella* using the sensitive Diassalm-Rambach technique (Davies 1997b; Davies, Bedford and Shankster, 2001b) at the VLA's Regional Laboratories (RLs). All isolates were serotyped and/or phagetyped and were reported as required by the Zoonoses Order 1989.

Data analysis

Data were entered on an MS Access database by staff of the Epidemiology Department, VLA Weybridge and statistical analyses conducted using Stataâ (StataCorp 2001 Stata Statistical Software. Release 7.0. College Station TX Stata Corporation USA). Farm-specific prevalence values for Visits 1 to 4 were calculated, with 95 per cent CIs using the Normal approximation or, for small sample size, an exact binomial distribution. Incidence rates, with exact 95 per cent CIs using a Poisson distribution, were calculated and expressed as cases per farm-years at risk.

Data extraction of passive surveillance data

All sample submissions to the VLA RLs are routinely recorded with various data relating to the type and source of the samples submitted (e.g. name, address, species of animal, etc.) on the VLA Farmfile database. A diagnosis is also recorded on the system where applicable. A *Salmonella* diagnosis should only be recorded on Farmfile when the organism is believed to be causing disease by the veterinary investigation officer. The majority of submissions to RLs are for diagnostic purposes. Submissions from 'healthy' livestock or samples submitted as part of a research project should have the diagnosis recorded as 'not applicable or screening'. *Salmonella* reports made under the Zoonoses Order 1989 are recorded on the Zoonoses database managed by the Epidemiology Department at VLA – Weybridge. The information collected and recorded includes details of the premises from which *Salmonella* was isolated, the species and age of the animal(s) involved, the type of sample taken and serogroup, serotype and phage type results as well as the associated Farmfile submission reference.

Zoonoses Order records and Farmfile submission data were matched using the Farmfile submission reference for incidents, as defined above, of *Salmonella* reported in cattle (excluding research data) in 2000 to produce the data set presented in this paper. The data was interrogated and compared with the data collected in the cohort study, for example to see whether all serotypes isolated in the cohort study were also detected by Statutory reporting of *Salmonella*. The regional distribution of *Salmonella* incidents reported by passive surveillance activities and the cohort study was also compared. An assessment of the level of under-reporting by passive surveillance was made based on the number of *Salmonella* incidents that were reported for cohort farms between study visits.

RESULTS

Cohort study

Enrolment and loss-to-follow-up

449 dairy farms were initially enrolled (48 (8.8 per cent) of 502 farms contacted were ineligible to join the study and 50 (9.1 per cent) declined). For logistical reasons, 362 farms were randomly selected to be followed up, and 24.9 per cent of these farms refused to participate and 30 farms were lost to follow-up. 243 (73.2 per cent of 332) farms were sampled at all four visits (Visits 1 – 4).

Salmonella prevalence

Estimates of *Salmonella* point prevalence ranged from 12.1 per cent (95 per cent CI: 8.2 – 16.0 per cent) at Visit 2 to 24.7 per cent (95 per cent CI: 19.4 – 30.1 per cent) at Visit 3 (Table 1). Farms were sampled during each month, although only five farms were sampled in February, and there was an observed trend of increasing Salmonella prevalence in late summer peaking at 34.8 per cent (95 per cent CI: 24.4 – 47.0 per cent) in August.

1 215 visits were carried out and a positive result was achieved at 232 of these visits (Table 2). Each of these positive visits can be considered as a separate incident based on the incident definition used for Statutory reporting. Over the

Table 1. Number and proportion of farms and samples with detectable *Salmonella* (all types) at each visit of the cohort study

Study visit (sampling period)	*No. of farm visits*	*No. of pooled samples*[1]	*Proportion (%) of samples with detectable Salmonella*[2]	*Proportion (%) of farms with detectable Salmonella*[2]	*95% CIs*[3]
1 (10/99 – 02/00)	449	7 299	4.8 (350)	18.7 (84)	15.0 – 24.4
2 (03/00 – 07/00)	272	4 225	3.3 (139)	12.1 (33)	8.2 – 16.0
3 (06/00 – 10/00)	251	3 869	5.8 (224)	24.7 (62)	19.4 – 30.1
4 (09/00 – 01/01)	243	3 903	4.9 (191)	21.8 (53)	19.3 – 24.3
All (10/99 – 01/01)	1 215	19 296	4.7 (904)	19.1 (232)	16.5 – 22.0[4]

[1] *Mean number of samples per farm ranged between 15-16 samples per farm at each visit*
[2] *Number of farms/samples shown in brackets*
[3] *CI: Normal approximation confidence intervals (not comparable between visits because of within-farm clustering) for proportion of farms with detectable Salmonella*
[4] *95 % CI adjusted for within-farm clustering*

study period 159 (35.4 per cent, 95 per cent CI: 31.0 – 39.8 per cent, adjusted for within-farm clustering) of the 449 farms were positive on a least one occasion. Seven (4.4 per cent) of these farms were positive at all four visits. 75 farms that were negative at Visit 1 became positive during the study and 132 farms were negative at all four visits (considered 'true negatives'). *S.* Dublin and *S.* Typhimurium were the only serotypes that were detected at all four visits on any farm and this occurred on a small proportion of farms only. A total of 29 serotypes of *Salmonella enterica enterica* were isolated during the study.

Salmonella incidence rate

The incidence rate was estimated to be 0.43 (95 per cent CI: 0.34-0.54) cases of (any serotype) per farm-years at risk. There was no significant difference between the incidence rates (cases per farm-years at risk) of the most common serotypes (*S.* Typhimurium, *S.* Agama, *S.* Dublin) found in the study.

Passive surveillance

All the serotypes isolated in the cohort study were also detected by Statutory reporting of Salmonella during the same period (Table 2) and include six of the ten most common serotypes reported in humans in England and Wales. Some serotypes (e.g. *S.* Virchow) were identified by Statutory reporting but not found in the cohort study.

A total of 1 035 *Salmonella* incidents were reported in cattle (on all farm types including dairy, beef or mixed farms) in 2000 in England and Wales, and, of these, 889 *Salmonella* incidents were reported, excluding all incidents where *Salmonella* was identified by research projects. In 2000 there were 95 104 submissions to VLA RLs, of which, 52 143 (54.8 per cent) were submissions

Table 2. Ten most common *Salmonella* serotypes found in humans[1] compared with those found in cattle through routine surveillance activities in 2000 and in the cohort study (*S.* Dublin and *S.* Agama incidents in cattle are also shown)

Serotype	*Humans*[1]		*Cattle*[2]		*Cohort Study Visits 1-4*	
	No. of isolations	*%*	*No. of incidents*	*%*	*No. of positive farm visits (incidents)*	*%*
Enteritidis	9 500	57.4	7	0.8	3	1.3
Typhimurium	2 982	18.0	196	22.0	37	15.9
Hadar	368	2.2	0	0	0	0
Virchow	333	2.0	1	0.1	0	0
Infantis	180	1.1	3	0.3	2	0.9
Newport	170	1.0	8	0.9	7	3.0
Blockley	164	1.0	0	0	0	0
Agona	162	1.0	6	0.7	17	7.3
Montevideo	114	0.9	3	0.3	1	0.4
Java	133	0.8	0	0	0	0
Dublin	-	-	580	65.2	58	25.0
Agama	-	-	29	3.2	55	23.7
Total	16 556		889		232	

[1] *Data from VLA 2000 Salmonella in Livestock Production in GB 2000*
[2] *Data extracted from VLA Farmfile database for year 2000*

Table 3. Regional distribution of *Salmonella* (*S.*) incidents reported in cattle through routine surveillance activities (all farm types) in 2000 and the cohort study (dairy farms)

Region	*No. Cattle herds*[1] *(%)*	*Salmonella incidents reported in 2000 (%)*	*Cohort study farms followed for all 4 visits (%)*	*No. S. incidents in cohort*[2] *(%)*	*No.S. incidents reported by cohort farms between visits*
North West	7 110 (9.5)	182 (20.5)	25 (10.3)	13 (7.2)	3
North East	9 637 (12.9)	40 (4.5)	17 (7.0)	7 (3.9)	1
Midlands	15 636 (20.9)	141 (15.9)	66 (27.2)	38 (21.0)	1
South E/East	10 870 (14.5)	45 (5.1)	34 (14.0)	22 (12.2)	0
South West	16 608 (22.2)	184 (20.7)	54 (22.2)	53 (29.3)	1
Wales	15 047 (20.1)	297 (33.4)	47 (19.3)	48 (26.5)	0
Total	74 908	889	243	181	6

[1] *Agricultural Census Data for England and Wales 2000 (includes all cattle farms) (National Statistics 2000).*
[2] *One farm could have up to 4 incidents as each positive visit counted as 1 incident*

from cattle. 43 714 (83.8 per cent) of all submissions were diagnostic submissions where samples were submitted by private veterinarians.

The 889 *Salmonella* incident reports from the VLA Zoonoses Order database were matched with their associated submission details on the VLA Farmfile database (Table 2 and 3). The animal purpose recorded on Farmfile was given as 'Milk' (i.e. dairy) for 574 (55.5 per cent) of the 1 035 *Salmonella*-associated cattle submissions. However, this is likely to be an under estimation of the actual number of dairy farms with incidents of *Salmonella* because for over 25 per cent of incidents, purpose was recorded as 'NA', 'None', 'Other', or 'Unknown'. It is likely that purpose was often not given for those farms with mixed enterprises. Over 70 per cent of the farms in the cohort study, which were all registered with a milk buying company, had other cattle types on the farm, suggesting that the majority of dairy farms have mixed cattle enterprises.

During the cohort study there were no Statutory *Salmonella* reports from any of the 132 true-negative farms. *Salmonella* was reported between sample visits by six of the 159 'positive farms' in the study (Table 3). Three farms had matching serotypes from Statutory reporting and the study (an additional serotype, *S.* Typhimurium RDNC, was found on one study farm) and three farms had mismatching serotypes (Statutory reporting – study results: 1) *S.* Typhimurium DT104 – S. 4,12:B-; 2) *S.* Enteritidis – *S.* Typhimurium DT104; 3) *S.* Typhimurium DT104 – *S.* Agama).

Almost 45 per cent of *Salmonella* incidents in 2000 were reported during the Autumn months (September – November) compared to 12 per cent of incidents reported during the Spring (March – May). Over 60 per cent of cattle farms were located in the Midlands, South West England and Wales (Table 3) and higher proportions of *Salmonella* incidents were reported in these regions. By contrast, only 9.5 per cent farms were located in the North West, but 20.5 per cent of all incidents were reported in this region. The regional distribution of the cohort dairy farms was similar to that of all cattle farms. The largest proportions of *Salmonella* incidents in the cohort study were observed in the South West and

Wales, but a lower proportion of incidents in the North West and a higher proportion of incidents in the South East/East were found in the cohort study compared with passive surveillance. These differences might reflect differences in submission rates to RLs in these regions.

DISCUSSION

The cohort study was the first study to estimate the prevalence of *Salmonella* in dairy herds in England and Wales and the results are comparable to findings of other studies, for example 12 – 28 per cent dairy herds were found to be infected with *Salmonella* in the USA (Smith *et al*, 1994). Overall 159 (35.4 per cent) of the 449 farms were *Salmonella*-positive on a least one occasion, but only seven (4.4 per cent) farms were positive at each visit. Seventy-five *Salmonella*-negative farms became positive giving an incidence rate of 0.43 (95 per cent CI: 0.34 – 0.54) cases of *Salmonella* (any serotype) per farm-years at risk. Based on the three-monthly environmental sampling protocol used, these findings suggest that 43 of 100 *Salmonella*-negative farms would be expected to become *Salmonella*-positive within a one-year period. Most *Salmonella*-infected cattle do not show clinical signs and therefore the majority of infections would not be detected by passive surveillance.

The proportion of positive farm visits (equivalent to incident reports) in the cohort study was comparable with the proportion of incidents recorded in cattle for each of the more commonly reported serotypes associated with human salmonellosis cases. Infected dairy farms have the potential to be a source of *Salmonella* for humans either through direct or indirect contact with cattle or the consumption of contaminated milk or meat products. *Salmonella* is rarely isolated from raw cow's milk, 0.3 per cent of 602 samples were positive in an 18-month study (DEFRA 2002), but people can also be exposed through the consumption of meat from young cull cattle or beef cattle reared on mixed enterprise farms (<10 per cent study farms were dairy only) or visiting farms (eg, open days).

Cattle are the principal host species for *S.* Dublin and the serotype rarely affects other livestock species or man. There have been increasing numbers of reports of *S.* Dublin infections in adult cattle and calves in recent years (VLA, 2000). Adult cattle can become life-long carriers of infection and therefore infection can be difficult to eradicate from endemically infected herds. The proportion of *S.* Dublin incidents identified through passive surveillance (diagnostic samples) in 2000 (58.2 per cent) was more than double the proportion of incidents identified during the cohort study. This difference is likely to reflect the increased likelihood of clinical disease in those cattle infected with *S.* Dublin and subsequent submission of samples. However, this serotype may persist less in the environment and thus is less likely to be isolated by the environmental sampling protocol employed during the cohort study.

In contrast, only 3.2 per cent of incidents reported under the Zoonoses Order were for *S.* Agama compared to 23.7 per cent of incidents identified during the

cohort study. *S.* Agama is mainly associated with cattle and sheep and rarely reported from human salmonellosis cases and is known to infect wildlife, including badgers. *S.* Agama had a high incidence rate and was more commonly found on farms with more than one serotype, possibly because of longer persistence in the environment and/or more sources of infection (e.g. wildlife). The comparison between the cohort study and passive surveillance data supports the evidence that *S.* Agama is less likely to cause clinical disease in cattle, because a lower proportion of *S.* Agama incidents were identified through passive surveillance. *S.* Typhimurium can infect a variety of host species, including humans (Hancock and others, 2000). Since the epidemic of multiple resistant *S.* Typhimurium DT 104 in the 1990's in GB, there have been declining reports of this Salmonella definitive type (VLA, 2001). The proportion of *S.* Typhimurium incidents identified through passive surveillance in 2000 (22 per cent) was slightly higher than the proportion of incidents with this serotype identified during the cohort study (15.9 per cent). Again this may indicate that cattle infected with this disease are more likely to have clinical disease and/or that this serotype is less likely to be isolated from environmental samples.

Differences in the serotypes isolated from environmental samples and diagnostic specimens has also been demonstrated by a study carried out in the USA in which the most common serotypes of *Salmonella* recovered from samples collected from feedlot pen floors were dissimilar from those most commonly associated with human illness or diagnostic specimens (NAHMS, 2001). Environmental sampling is a cost-effective method which enables large-scale studies to be conducted to determine herd-level prevalence. However, it would appear to identify different (relative proportions of) serotypes than individual animal samples and whilst it could be useful as a screening test for some serotypes (e.g. *S.* Typhimurium), it may be less useful for other studies depending on their purpose.

The cohort study farm population was considered to be representative of the general dairy farm population in England and Wales. During the study there were no Statutory reports for any of the 132 farms that were negative at each visit, but only six of the 159 *Salmonella*-positive farms also had Statutory *Salmonella* reports and in half of these farms the same serotype was identified. The regional distribution of study farms was similar to the distribution of all cattle herds in England and Wales. The highest proportions of *Salmonella* incidents were reported in the Midlands, South West of England and Wales by both the cohort study and passive surveillance. However, the proportion of incidents reported in the cohort study in the South East/East of England was higher, and in the North West was lower, than the proportions of *Salmonella* incidents reported by passive surveillance. The fact that only six of the 159 positive farms reported *Salmonella* incidents between study visits suggests that only one in 26 *Salmonella* incidents may actually have been reported through passive surveillance, although submission rates may have been biased (higher or lower) by participation in the study. The data suggest that the degree of under-

reporting varies by region and further work will examine these differences more fully. Spatial analysis techniques could be applied to assess the distribution of cohort farms and farms that submit samples to RLs in selected regions. Information on these distributions would allow comparison between the study results and passive surveillance reports to more accurately estimate the level of under-reporting. The seasonal distribution of *Salmonella* incidents reported during the cohort study showed a very similar pattern to reports made through passive surveillance activities, with the highest proportion of incidents reported in the autumn.

Comparisons such as these presented here are useful to assess existing surveillance systems and identify where enhanced or targeted surveillance activities are required. In the future, active surveillance and the development of early warning systems (e.g. for emerging *Salmonella* serotypes) will be increasingly important.

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DESIGN AND IMPLEMENTATION OF A MONITORING SYSTEM FOR WEST NILE VIRUS IN HORSES IN THE SOUTH OF FRANCE

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SUMMARY

Disease monitoring is becoming one of the major components of veterinary activity and a prerequisite for effective disease control (Anonymous, 2000). Recent outbreaks underline the importance for reliable monitoring systems capable of early identification of emerging diseases. The Centers for Disease Control (CDC) in the USA identified good surveillance and prompt response as the first of four major goals in modern strategies for preventing emerging infectious diseases (Anonymous, 1998).

'Veterinary surveillance' has been defined as the systematic collection of data on the occurrence of specific diseases, the analysis and interpretation of these data, with timely dissemination of that information to those responsible for control and prevention measures (Dufour and Audige, 1997). Usually, the term 'monitoring' is used for a continuous, dynamic process of collecting data about disease, but without any immediate control activities (Doherr and Audige, 2001). We like to use the term of monitoring rather than surveillance in our presentation.

The aim of this work is to summarise and discuss some methodological issues related to veterinary animal health monitoring. We will illustrate this using the recent outbreak of West Nile virus that occurred in France in 2000 as an example, knowing that the first reported outbreak occurred during the summer of 1962 (Joubert, Oudar *et al*, 1970).

BACKGROUND

West Nile Disease

West Nile (WN) fever is a mosquito-borne flaviviral infection transmitted in natural cycles between birds and mosquitoes, particularly Culex species (Murgue, Murri *et al*, 2001). The epidemiological cycle of West Nile virus is presented in Figure 1. Largely bird-feeding species mosquitoes are the principal vectors of West Nile virus. Infection can cause febrile, sometimes fatal, illnesses in horses, birds and humans. Wild birds are the principal hosts and humans and horses usually represent incidental and dead-end hosts (Komar, 2000).

Natural foci of West Nile virus infections are mainly situated in wetland ecosystems (river deltas or flood plains) and are characterized by the bird-mosquito cycle. In Europe, West Nile virus circulation is confined to two basic types of

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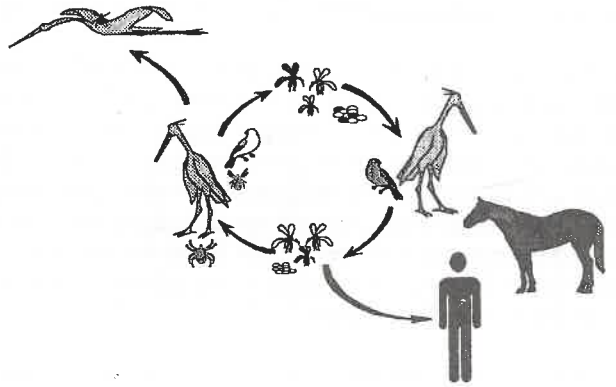
cycles and ecosystems (Komar 2000):

- rural (sylvatic) cycle: wild, usually wetland birds and ornithophilic mosquitoes,
- urban cycle: synanthropic or domestic birds and mosquitoes feeding on both birds and humans, mainly *Culex pipiens/modestus*.

Figure 1. Schematic diagram of the epidemiologic cycle of West Nile virus (Zeiler 2001)



The virus has been isolated from 43 mosquito species, predominantly of the genus Culex. Infection ofarthropods is lifelong. The mechanism of West Nile virus persistence in diseaseendemic foci of temperate Europe represents a challenge for further research. General hypotheses of overwintering or ovarial transmission are currently under evaluation.

Birds usually do not show any symptoms when infected. Occasional encephalitis, death or long term persistence of the virus have been reported. High, long term viremia, sufficient to infect vector mosquitoes has been observed. Migratory birds are therefore instrumental in the introduction of the virus to temperate areas during spring migrations (Hars, 2001).

Affected horses exhibit biphasic fever signs, followed by encephalomyelitis with staggering gait, hind limb weakness and paralysis, often leading to recumbency and death (Komar, 2000). The frequency of unnoticed infection in association with cases of clinical disease is difficult to establish. Mammals are less important for maintaining transmission cycles in ecosystems.

The first European isolations of the virus were recorded in 1963, from patients and mosquitoes in Rhone Delta, and from patients and ticks (*Hyalomma marginatum*) in the Volga Delta. West Nile virus was subsequently isolated in Portugal, Slovakia, Moldavia, Ukraine, Hungary, Romania, Czechland and Italy (Hubaiek and Halouzka, 1999).

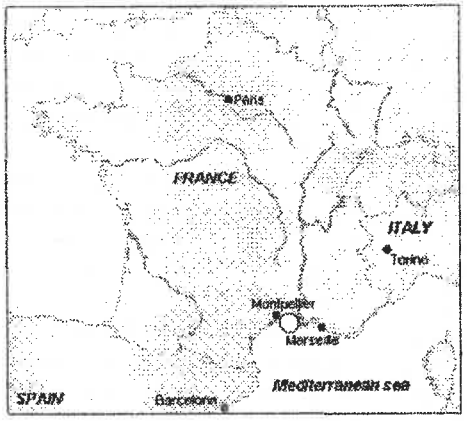
METHODS AND RESULTS

West Nile disease outbreak, France 2000

A short background on the recent outbreak of West Nile virus that occurred in southern France in autumn 2000 is worthwhile to understand the present evolution. Until the year 2000 a passive monitoring system was in place. A passive monitoring system is commonly defined as the report of suspect cases to the health authorities by health care professionals at their discretion (Dufour and Audige, 1997). These systems rely on a pyramid of scrutiny which includes the animal owners, private practitioners and veterinary laboratories to form the three levels.

The system allowed the report of two cases of West Nile encephalitis in horses on 6 September 2000 (Figure 2). Through 30 November 76 cases were laboratory confirmed among 131 equines with neurologic disorders and 21 horses died. The last confirmed case was on 3 November 2000 (Murgue, Murri *et al*, 2001) . Clinical responses in horses ranged from no apparent illness to fulminating fatal encephalitis. The most common signs were symmetric or asymmetric limb ataxia or paresis. This more commonly involved the rear limbs, but was occasionally limited to the front limbs. Muscle fasciculations and tremors involving the face, lips, trunks or shoulder were seen (Zientara, 2001).

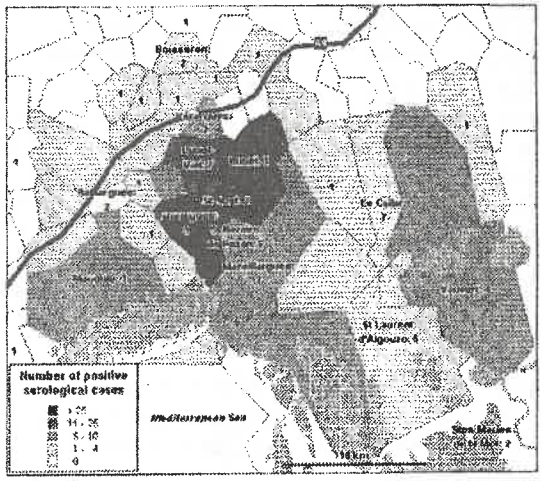
Figure 2: Geographic location of horses with laboratory-confirmed West Nile virus infection, France 2000 (Murgue 2001)



All but three confirmed and probable cases were located within an area of 15 km radius, in a region ofHerault and Gard provinces called 'La Petite Camargue', in southern France (Figure 3) (Durand, Chevalier *et al*, 2002). Three cases, all fatal, were located near this area, in Bouches du Rh6ne province, about 30km from the first reported cases, near the Camargue National Park, were a West Nile outbreak occurred in 1962. This is the delta of the Rhone river, were there is a

large population of feral horses. The region is characterized by its original flora (wetlands, ricefields) and fauna: more than 300 migratory and resident bird species and large populations of mosquitoes. No abnormal deaths of birds were reported. Thousands of horses live in this region. The WN vector species in the present outbreak is still unknown.

Figure 3. Geographic location of confirmed and probable clinical cases and serological positive cases of West Nile virus infection in equines, France 2000 (Murgue 2001)



After the recognition of the outbreak, an assessment of the underestimation of the disease has been attempted by initiating a large serosurvey on all equids located within a ten km radius of laboratory-confirmed and probable cases. Blood samples were obtained from 5 107 equines. West Nile lgG antibodies were found in 8.5 per cent of animals. While there was no age effect in the seroprevalence, a strong group size effect was found, horses living in small groups being more affected than those located in large groups. The geographic variations of the seroprevalence showed the existence of a hotspot located in a dry area (Durand, Chevalier *et al*, 2002).

These results suggested two types of hypotheses that could explain the observed outbreak of West Nile virus:

- An area of endemic West Nile disease exists in some humid zone, near the area of the outbreak. For some unknown reasons (climatic conditions, for example), which lead to the emergence of an epidemic in the year 2000.
- The disease was introduced by migrating birds. A local epidemiological cycle developed then between local non migrating birds and mosquitoes and allowed the spread of the disease in non-humid zones.

These hypotheses were taken into consideration for the design and implemen-

tation of the monitoring of the disease during the following year.

West Nile monitoring in 2001

Disadvantages of passive monitoring systems are that subclinical infections are not recognized, and the case-reporting level can sometimes be low (Doherr and Audige, 2001). During the year 2001, both passive and active monitoring systems for West Nile disease were implemented. Active monitoring has been defined as the regular periodic collection of case reports from health-care providers or facilities (Dufour and Audige, 1997). In contrast to passive monitoring, individuals to be included during active monitoring are selected through a formal sampling process, that, in theory, should select each likely individual. Alternatively, a target population can be defined, depending of the condition of interest, the expected prevalence or the risk factors influencing the distribution of the disease (Doherr and Audige, 2001).

The objectives of the system were:

- to evaluate the persistence of the virus in the populations of birds and mosquitoes in the region of the outbreak 2000,
- to identify early in the season the presence of virus in regions of Camargue other than those affected in 2000.

The system was passive for horses and humans, with enhanced awareness of practitioners of clinical signs of the disease. Active monitoring of mosquitoes was funded by health authorities too, with protocols of capture and PCR analysis on pools of mosquitoes.

For birds, both passive and active monitoring systems were implemented. A passive system existed already in France for the monitoring of the causes of mortality in wild birds (SAGIR network). The monitoring was enhanced by the wide circulation of information on West Nile disease in the population and the secondment of a call free-number to allow the reporting of dead bird cases. Active monitoring of birds consisted of serological surveys on duck farming, considered as sentinels. Blood was taken from these birds once a month from July to November. Otherwise, serological testing was done on wild birds (magpies, *Pica pica* and pink flamingos, *Phoenicopterus rubber*) caught in August and September (Hars, 2001). Finally 28 magpies, 109 first-year flamingos, 178 ducks and 129 fowls, distributed in 30 distinct geographic locations, were tested.

Last, this system allowed the detection of a seroconversion on one duck that became positive on 10 October 2001, near the town of 'Arles'. The major disadvantage of such active monitoring systems is that it becomes very costly when the target disease becomes rare. It can be shown that sample sizes, and therefore costs, increase exponentially with a decreasing prevalence to detect (Doherr and Audige, 2001). The aim of the surveys then changes from estimating a low prevalence to the assumption of disease free and therefore the identification of a health-related event if it occurs above a threshold prevalence (generally set to 0.1 or 0.2 per cent) (Cameron and Baldock, 1998a; Cameron and Baldock, 1998b).

Perspectives

In the light of these results, objectives of the monitoring system were redefined. Rather than monitoring the outcomes itself (disease or health related event) the system should monitor the risk factors for the disease. These objectives should allow one to identify potential high-risk populations and estimate the risk of re-emerging disease in a define geographic area. The principle of the system lies on the hypothesis that there is a close relationship between environmental factors, biology of the vector and emergence of the disease. These principles are applied for collecting disease information from a range of sources and building forecast models. These models are used to explain observed phenomena and estimate the probability of emergence of the disease in a given population at a given time.

The design of a model is mainly influenced by our understanding of the mechanisms of transmission of the disease. Regarding the WN virus transmission cycle, we know that mosquitoes, largely bird-feeding species, are the principal vectors of WN virus. We can reasonably assume that one relevant indicator for estimating the risk of disease is the abundance of mosquitoes as vectors. The West Nile virus transmission cycle could be modified under certain climatic conditions. For example, during a mild winter, more mosquitoes than usual survive. A lack of rain during a dry spring and summer kills predators of mosquitoes and congregations of birds at water sources are observed. At last, a heat wave in July (wet season) allows a rapid proliferation of the virus in the vector, and the beginning of a vicious cycle with emergence of a possible epidemic outbreak (Epstein, 2001).

To take into account the climatic factors that can influence the populations of mosquitoes, we began to design a model to estimate the dynamic variations of water level in a ground pool (the main vector habitat) in relation with rainfalls. The main inputs are represented by rainfall. The ways for water level depletion are mainly evaporation and infiltration which are the two mechanisms for emptying. So, we could have an anticipation of the water level in the ground pool, using rainfall variations during a given month. The second step was to estimate the abundance and the peak of abundance of mosquito populations. The exact mosquito species involved in the recent outbreak in France is still unknown, but it is generally accepted that a *Culex* spp. may play a major role (Hannoun, Panthier *et al*, 1969). So, if we take *Culex* spp. as example, we know that it lays around 200 eggs on the surface of the water, that larval and nymphal stages live one to three weeks in the water, and that adults can survive one month (Hayes, Basit *et al*, 1980). Thus, it was possible to anticipate the abundance and the peak of adult mosquitoes, related to the occurrence of rainfalls for a given month. Third step was the characterisation of monthly and inter-annual variations of rainfall, and then the model could estimate the abundance of adult mosquitoes for a given year.

Next, we have to compare the results of the model and to estimate its sensitivity with observed data, collected through the monitoring system. For the WN virus monitoring, we defined a geographic area that comprises 'the little Camargue' (east of Montpellier), the large Camargue (south of Nîmes), and 'le plan du Bourg' (west of Arles and Marseille). Data collected could be grouped

into three categories: animal, vector and environmental data.

Data on animal populations are collected through the implementation of a passive/active monitoring system. Veterinary sentinels are equipped with modern tools of communication like, for example Palm Pilots and Internet access. The Palm Pilots allow the collection of the data in the field and their transmission in real-time. Furthermore, certain indicators showing possible emergence can allow health authorities to respond appropriately.

Environmental data, for example climatic data, are obtained by the 'bioclimatology' laboratory of INRA, in Avignon. The characterisation of the biotope is made through studies on the ground and their correlation established with satellite imaging. The different colours observed in the SPOT 4 images (HR VIR : High Resolution, Visible Infra-Red, Decades) from the Camargue region allow one to categorize different biotopes, wetland areas and vegetation, fields and urban zones. Analysis of such imaging, combined with climatologic data could provide an estimation of the populations of vectors, even at a small scale.

Other categories of data on vector populations are collected. An organism called 'EID' (Organisation for clearing of mosquitoes in the Mediterranean) is funded by the Ministry of environment to control the populations of mosquitoes that could be deleterious for the touristy activity. This organisation provides maps of larval sites and estimates of adult populations of health-related species of mosquitoes.

CONCLUSION

Such systems require the collaboration of several specialists. As for natural disasters, it becomes necessary to design monitoring systems capable of estimating the risk of emergence through the continuous monitoring of relevant indicators. Such systems need to be constructed and should be constantly updated.

ACKNOWLEDGEMENTS

This work was conducted thanks to a cooperative agreement between some French organisations, grouped under the consortium called Spatial Surveillance of Epidemics, and the funding of the 'Haras Nationaux', the laboratories 'Mérial' and AFSSA (French Agency of Food Hygiene and Security).

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HOW TO SUBSTANTIATE ERADICATION OF BOVINE BRUCELLOSIS WHEN ASPECIFIC SEROLOGICAL REACTIONS OCCUR IN THE COURSE OF BRUCELLOSIS TESTING

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SUMMARY

Collaborative work was financed by the EU to develop and assess new diagnostic tools that can differentiate between bovine brucellosis and bovine infections due to *Yersinia enterocolitica* O: 9 either in conjunction with, or, as an alternative to, the classical serological, bacteriological or allergic skin tests. Sixteen heifers were experimentally infected with *B. abortus* biovar 1 (five hefers), *B. suis* biovar 2 (two heifers), *Y. enterocolitica* O:9 (six heifers) and *Y. enterocolitica* O:3 (3 heifers). Four heifers, naturally infected with *Y. enterocolitica* O:9 that presented aspecific brucellosis serological reactions were also included in the experiment. A self-limited infection was induced in cattle by *B. suis* biovar 2. All the brucellosis serological tests used, i.e. the Slow Agglutination Test, the Rose Bengal Test, the Complement Fixation Test, Indirect and Competitive ELISA's, lacked specificity when used to analyze sera from *Y. enterocolitica* O:9 infected animals. A YOPs-ELISA (*Yersinia* Outer Membrane Proteins) was also used and, although the test is able to detect a *Yersinia* group infection, it provided no evidence of whether or not there is a possible brucellosis infection when dual infections are present. The brucellergen IFN-*γ* test showed a lack of specificity also. The only test that was proven to be specific is the brucellergen skin test. All brucellosis serological tests, except the indirect ELISA, were limited in their ability to detect *B. abortus* persistently infected animals.

Based on these experimental studies, a strategy was implemented as part of the year 2001 Belgian Brucellosis Eradication Program to substantiate the eradication of bovine brucellosis. Epidemiological inquiries have identified risk factors associated with aspecific serological reactions, possible transmission and

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infection of cattle by *B. suis* biovar 2 from infected wild boars; and both legal and administrative measures taken by the veterinary services. No cases of bovine brucellosis have been confirmed in Belgium since March 2000.

INTRODUCTION

In the European Union (EU), bovine brucellosis due to *Brucella abortus*, has been eradicated in some of the 15 Member States, according to EU Directive 64/432/EEC, modified by EU Directive 97/12/CE. Denmark (Decision 79/837), Finland (Decision 94/960), Germany (Decision 80/775), Sweden (Decision 95/74), United Kingdom (Great Britain) (Decision 97/175) and since 1999, Luxemburg, The Netherlands, Austria and Italy (Province of Bolzano) (Decision 99/466/EEC) are recognised as 'officially free from bovine brucellosis'. Nevertheless, cattle herds continue to be serologically monitored for the potential re-emergence of the disease. While for some other EU Member States the outlook for eradication is very favourable (no brucellosis case reported for the last two years in Belgium, for example) for other EU Member States it is not so favourable due to an unacceptable level of prevalence of infection in certain regions of these EU Member States. Within the EU a Community Reference Laboratory (CRL) for the epidemiology of zoonoses has been designated at the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany. The CRL is responsible for co-ordinating the reports of the National Reference Laboratories of the Member States and providing the Member States with an annual report. The *Report on Trends and sources of zoonotic agents in the European Union and Norway, 2000* is available upon request to the CRL for the epidemiology of zoonoses. Information about brucellosis (world wide) is available on the OIE website (http://www.oie.int), by using the HandiStatus tool (i.e. Help with World Animal Disease Status - version 2) containing information on animal diseases (like brucellosis) that have serious consequences for international trade or public health. This information is regularly updated based on the emergency, monthly and annual reports sent to the Central Bureau of the OIE by the veterinary administrations of countries and other official sources.

The diagnosis of bovine brucellosis is based almost exclusively on serological tests. Trade in non officially brucellosis free Member States is allowed only if animals originate from a 'brucellosis officially free herd' and if animals are classified negative in serological pre-movement tests. These tests have proved to be specific and sensitive enough for use in a test-and-slaughter eradication programme. However, when an eradication program is close to its end, a high proportion of positive serological results may be obtained even in the absence of brucellosis infection, because brucellosis serological tests are 'imperfect tests'. For example, if the Rose Bengal test (assuming a sensitivity of 75 per cent and a specificity of 99.2 per cent) is applied in a cattle population where the prevalence rate is one per cent, the positive predictive value (the probability to be a

true positive, i. e. infected, testing positive) is estimated to be 48 per cent. But, if the same test is applied in a population where the prevalence rate is 0.1 per cent, then the positive predictive value drops to eight per cent, meaning that 92 per cent of the animals classified sero-positive are actually not infected (Godfroid, 1992). Hence, in a population free of the disease, the positive predictive value of a serological test is, by definition, zero. Therefore, better tests, as well as a testing strategy adapted to the epidemiological situation, need to be developed and validated.

Although a certain number of problems were expected at the closing stages of eradication programs in the EU, a high proportion of non specific reactions relative to the total of all brucellosis serological tests used as official tests in the EU have emerged throughout Europe. This has been documented since early 1990 in Belgium and France, affecting up to 15 per cent of the herds tested in some regions that are free of the disease (Gerbier *et al*, 1997; Saegerman *et al*, 1997; Pouillot *et al*, 1998). In those cases, in-depth epidemiological inquiries, as well as the absence of isolation of *B. abortus* in seropositive animals, have proved the absence of brucellosis in such herds. However, *Yersinia enterocolitica* O: 9 (YO9), which shares common antigenic epitopes with *B. abortus* (BA), has been isolated in numerous cases (Weynants *et al*, 1995; Weynants *et al*, 1996; Gerbier *et al*, 1997; Pouillot *et al*, 1998). The immunodominant O-chain of smooth LPS (S-LPS) of both *Y. enterocolitica* O:9 and *B. abortus* contains common antigenic epitopes detected in the brucellosis serological tests using whole bacteria or S-LPS extracts (Corbel, 1985). On the other hand, the cell-mediated response in bovine brucellosis is directed against proteins and can be measured by the brucellosis skin test or the brucellosis IFN-γ test, using a mixture of cytoplasmic proteins commercially available (brucellergen, Synbiotics) (Weynants *et al*, 1995, Saegerman et al, 1999). However, since its first use, the brucellosis IFN-γ test has been shown to be less specific than expected (Kittelberger *et al*, 1997). On the contrary, the brucellosis skin test has consistently proven to be a very specific test (Mc. Diarmid, 1987, Pouillot *et al*, 1997, Saegerman *et al*, 1999).

It has been shown that when *B. abortus* is eradicated in a cattle population, *B. melitensis* or *B. suis* may infect cattle, the disease being indistinguishable from *B. abortus* infection (Corbel, 1997). In the EU *B. melitensis* induced abortions in cattle that were in contact with infected sheep (Verger *et al*, 1989). Recently, brucellosis in wild boars (*Sus scrofa*) due to *B. suis* biovar 2 has been described in Belgium (Godfroid *et al*, 1994) and seems to be widely distributed among wild boars as well as in hares (*Lepus capensis*) throughout Europe. Frequent contact between wild boars and cattle has been reported and the risk of cattle being exposed to *B. suis* biovar 2 has to be assessed. *B. suis* biovar 1 infections have been reported in cattle and with it excretion of Brucellae in cow's milk, limited induced pathology and no induction of abortion (Cook *et al*, 1984; Ewalt *et al*, 1997). Although *B. suis* biovar 2 was isolated from cattle in Denmark at one time (Andersen *et al*, 1995), the pathogenicity of *B. suis* biovar 2 in cattle is actually unknown.

This manuscript reviews the potential of different tests to differentiate between bovine brucellosis and experimental infections due to *Y. enterocolitica* O:9; and documents the strategy currently applied in Belgium to substantiate the eradication of bovine brucellosis in the cattle population. Based on those results, new testing strategies have been developed and validated (Jacobson, 1997) in Belgium and France.

MATERIALS AND METHODS

Animals and Experimental infection

Sixteen Holstein non-pregnant heifers (12-14 months of age) were purchased in three different herds, in a brucellosis free area. The herds of origin were classified 'brucellosis-free herds' and no positive serological results had been documented in these herds for the past ten years. Heifers were divided randomly into four different groups that were housed in different stables to avoid possible cross-contamination.

- Group 1: Four heifers infected with *B. abortus* biovar 1;
- Group 2: Two heifers infected with *B. suis* biovar 2;
- Group 3: Six heifers infected with *Y. enterocolitica* O:9;
- Group 4: Three heifers infected with *Y. enterocolitica* O:3.

In addition, four heifers that presented aspecific serological reactions during the compulsory brucellosis screening program were included in the experiment (Group 5) in order to follow-up the kinetic of these serological reactions. Two of these animals were shedding *Y. enterocolitica* O:9 in their faeces when they were included in the experiment.

The immune status of all animals was determined weekly by serology and IFN-γ tests over an eight week period. In addition, Castaneda blood cultures were performed weekly for animals from Group 1 and 2. Faecal excretion of *Y. enterocolitica* O:3 or O:9 was determined weekly for animals from Groups 3, 4 and 5 and, on three occasions for animals from Groups 1 and 2, in order to monitor for cross-contamination.

Bacterial strains

Five animals were infected with 5×10^6 of the *B. abortus* biovar 1 reference strain 544 (ATCC n°235448) by the conjunctival route. Two animals were infected with 10^6 of the *B. suis* biovar 2 Belgian field strain S120 by the conjunctival route and re-infected with 3×10^8 subcutaneously, two months later (day 0 of the experiment). Six heifers were infected with 10^{12} *Y. enterocolitica* O:9 French field strain 94/7053 by the oral route, three days consecutively. This strain was identified, biotyped and serogrouped by the classical methods (Bercovier *et al*, 1980; Wauters, 1981; Wauters *et al*, 1987). This strain harboured the *Yersinia* virulence plasmid pYV, as confirmed by the calcium dependency test (Gemski *et al*, 1980). Three heifers were infected by the oral route, three days consecutively, with 10^{12} *Y. enterocolitica* O:3 strain WE261/87 pYV+

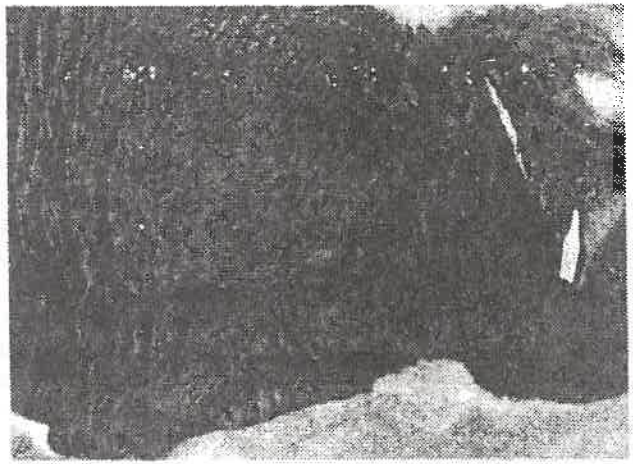
(obtained from the Department of Microbiology, University Hospital St Luc, Belgium), harbouring the *Yersinia* virulence plasmid pYV as confirmed by the calcium dependency test (Gemski *et al*, 1980).

Cell Mediated Immunity assessment

The brucellosis IFN-y test was performed weekly as previously described (Weynants et *al*, 1995). Briefly, blood was collected from the jugular vein by using preservative-free sodium heparin as an anticoagulant. Samples were dispatched to the laboratory within eight hours. Stimulations were performed in duplicate by mixing in tubes (Micronic, the Netherlands) 1 ml of blood and either 100 µl of phosphate-buffered saline (PBS) (negative control) or antigenic stimuli (brucellergen at 40 µg per well). The culture was incubated for 18 to 24 hours at 37°C in a humidified atmosphere with five per cent CO^2. Supernatants were then harvested and stored at -20°C until assayed for IFN-y content. IFN-y was assayed by using an ELISA kit (Biosource, Belgium). The test was performed according to the manufacturer's instructions. For a given animal, results were expressed in stimulation indices (SI) by using the following formula: mean of the optical density (OD) of the culture with antigen divided by the mean of the optical density of the control culture. A culture was considered to produce a significant level of IFN-y if the SI was equal to or greater than 2,5. If the OD of the control culture was equal to or greater than 0.150, i.e. producing a significant amount of IFN-y without addition of antigen, the test was considered as non-interpretable.

In order to perform a valid IFN-y test, the brucellosis skin test (Saegerman *et al*, 1999) was only performed at the end of the experiment. Briefly, the test was

Figure 1. Positive skin test (reading 72 hours after intra dermal injection of 100 µl brucellergen) following exposure to brucellae



performed by intradermal injection of 100 µl of brucellergen into a previously shaved skin fold at the side of the neck. The thickness of the skin fold was measured with a spring skin meter (Aesculap, Germany) just before and 72 hours after the injection. A thickening greater than 1.1 mm was considered positive. In both the IFN-y test and brucellosis skin test, the same batch of brucellergen (batch n°172) was used.

Brucellosis Serology

Classical serological tests i.e. the Rose Bengal Test (RB), the Slow Agglutination Test (SAW) and the Complement Fixation Test (CFT), were preformed as described in techniques for the brucellosis laboratory (Alton *et al*, 1988) and in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Anon, 2000) and interpreted according to EU directive 64/432/EEC.

An indirect ELISA described previously (Limet *et al*, 1988) was used. This ELISA fulfils the requirement laid down in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Anon, 2000).

A specific competition ELISA (cELISA) has been developed based on a monoclonal antibody (BM40) to an epitope (M-epitope) present in the *Brucella* LPS but absent from *Y. enterocolitica* O:9 LPS (Caroff *et al*, 1984). The results are expressed as a percentage of the negative control serum included on each plate. In order to be considered positive, a reduction of 30 per cent of the negative control serum was required.

Yersinia serology

An indirect YOPs-ELISA (Yersinia Outer membrane Proteins), described previously (Weynants *et al*, 1996), was performed. The 4-parameter logistic equation, from the Delta Soft software developed by Biometallics (Princeton, USA), was used to convert the OD value of each serum sample using the standard curve and convert it into arbitrary units.

Bacteriology

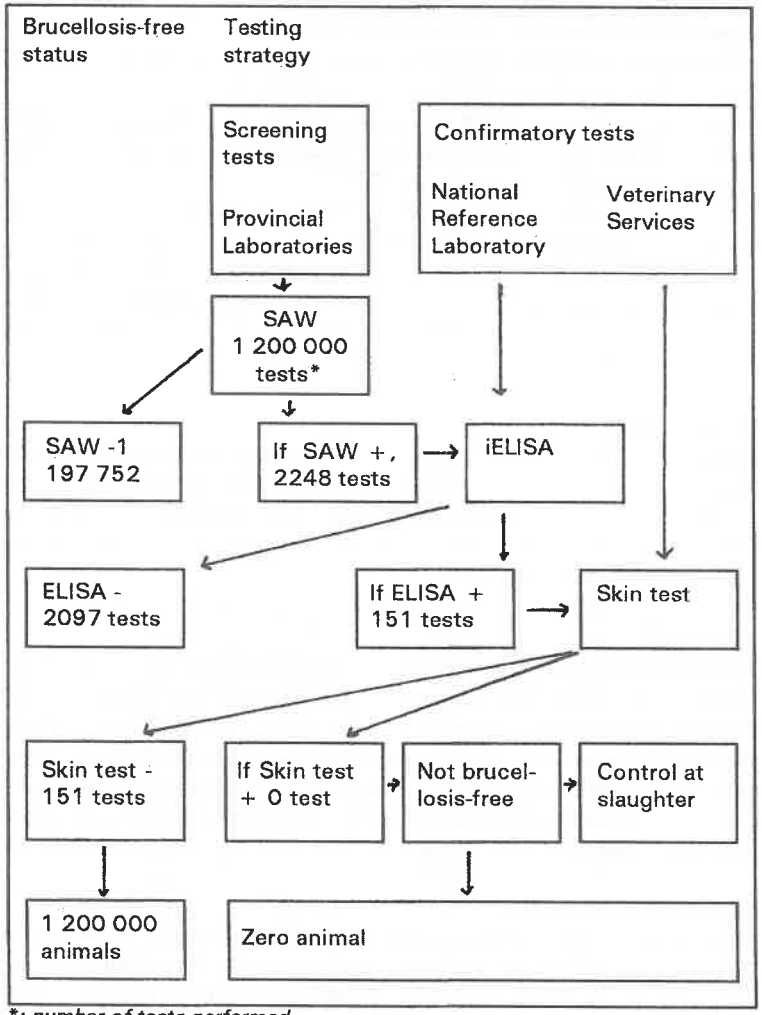
Blood cultures (weekly) and pertinent organs (sampled at slaughter) from the *B. abortus* and *B. suis* infected animals were subjected to bacteriological examination, according to standard protocols (Alton *et al*, 1988). For *Y. en-terocolitica*, faecal samples were plated after an enrichment incubation of the sample suspensions in PBS for seven days at 4°C. Isolation of *Y. enterocolitica* O:3 and O:9 was performed after incubation on a CIN Yersinia selective solid medium (Biomérieux, France).

Field animals and samples

In Belgium out of a population of three million cattle, about 1 200 000 animals (more than two years of age) are serologically tested for brucellosis every year. The last case of brucellosis (confirmed by the isolation of *B. abortus* biovar 3) was found in March 2000. Based on these experimental results, a testing strat-

egy has been proposed to the Belgian veterinary services in order to (1) substantiate the eradication of brucellosis and to (2) deal with aspecific serological reactions. On those animals that tested positive in the brucellosis screening test, i.e. the SAW, a 'decision tree' was initiated in the year 2000 eradication program and applied to a limited number of cases and then, as part of the official 2001 Belgium Eradication Program, implemented throughout the whole country.

Figure 2: Decision tree applied in case of SAW seropositivity during the 2001 Belgian brucellosis eradication campaign



*: number of tests performed

RESULTS

Experimental infection

The brucellosis serological results are shown in Tables 1 to 5. The *Y. enterocolitica* O:9 infection induced high and persistent antibody titers in all tests, whereas, as expected, no positive reactions was observed for the *Y. enterocolitica* 0:3 infected group. The most striking result is that when young heifers were infected with *B. abortus* biovar 1 at a dose that is one log less than the dose known to induce certain abortion in pregnant heifers (Fensterbank, 1987), none of the tests could identify all infected animals, although one week p.i, one of four animals tested positive with the SAW, RB and iELISA. Moreover, the CFT that is widely used as a confirmatory test after a positive result is obtained either by the RB or the SAW test, detected only 2/5 infected animals 7 W p.i (Table 3). On the other hand, the CFT, considered a very specific test, classified a majority of *Y. enterocolitica* O:9 infected animals as positive. *B. suis* biovar 2 also induced positive reactions in serological examination of 2/2 animals. However, it is important to note that these animals were infected twice and that after the first conjunctival infection serological tests were negative even after two months. Thus, the animals were re-infected sub-cutaneously on day 0.

The brucellosis IFN-y test appeared to be less specific than first reported in field conditions (Weynants *et al*, 1995). It has been reported that after oral exposure to *Y. enterocolitica* O:9, the brucellergen-stimulated release of IFN-y peaked at values above the cut-off stimulation index of 2.5 in 80 per cent of the heifers. (Kittelberger *et al*, 1997). Although this percentage was not obtained in this study for both Groups 3 and 4, some animals were inconsistently classified as positive in this test, as shown in Table 6. Further, the test showed a lack of sensitivity at the animal level.

The YOPs-ELISA classified animals of Groups 3 and 4 correctly as well as 2/4 animals that presented aspecific positive serology during the brucellosis screening campaign. However, one animal infected with *B. abortus* biovar 1 was classified positive by the YOPs-ELISA and could later have been classified non infected with *B. abortus* biovar 1 (Table 7).

The *Y. enterocolitica* bacteriological results are shown in Table 8. No cross-contamination among different groups could be detected. Although, *Y. enterocolitica* O:9 was shed in the faeces for more than eight weeks in 2/6 animals, positive serological results were obtained without isolation of *Y. enterocolitica* O:9 from the faeces of a given animal, for more than six weeks. Two of three animals infected with *Y. enterocolitica* O:3 shed bacteria throughout the experiment, supporting the serological results.

The weekly blood cultures performed on Groups 1 and 2 never resulted in the isolation of brucellae. At slaughter *B. abortus* biovar 1 was isolated from 3/5 infected animals. Brucellae were recovered from the tonsils and the retro-mandibular, parotid and mammary lymph nodes but not from the spleen and the uterus. *B. abortus* biovar 1, could not be isolated from 2/5 animals. We therefore

concluded that they cleared the infection. A combination of RB, SAW and CFT would have missed one infected animal in all circumstances. Only the indirect ELISA identified correctly these three persistently infected animals, six weeks p.i. *B. suis* biovar 2 could not be isolated in animals of group 2. We thus concluded that these cattle cleared the *B. suis* 2 infection even when given a high dose.

The brucellosis skin test was performed on all animals at the end of the experiment. None of the animals of groups 3, 4 and 5 presented a positive reaction. The three persistently *B. abortus* biovar 1 infected animals were classified as positive (Figure 1), whereas the two animals that had cleared the infection were classified as negative. Both *B. suis* biovar 2 infected animals were classified positive, although they had cleared the infection at the time the skin test was performed.

Table 1. Number of animals per group classified positive by the Slow Agglutination Test (SAW)

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	1	1	1	1	1	2	2	2
BS (2)	0	1	2	2	2	2	1	1	1
YO9 (6)	0	1	6	5	5	5	4	2	1
YO3 (3)	0	0	0	0	0	0	0	0	0
FPSR (4)	1	1	0	0	0	0	0	0	0

( )°: number of animals per group

Table 2. Number of animals per group classified positive by the Rose Bengal (RB)

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	1	1	1	1	1	2	2	2
BS (2)	0	1	2	2	2	2	1	1	1
YO9 (6)	0	1	6	5	5	5	4	2	1
YO3 (3)	0	0	0	0	0	0	0	0	0
FPSR (4)	1	1	0	0	0	0	0	0	0

( )°: number of animals per group

Table 3. Number of animals per group classified positive by the Complement Fixation Test (CFT)

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	0	0	0	0	0	0	2	2
BS (2)	0	0	1	1	1	1	1	1	1
YO9 (6)	0	0	5	6	6	5	4	5	5
YO3 (3)	0	0	0	0	0	0	0	0	0
FPSR (4)	1	1	0	0	0	0	0	0	0

( )°: number of animals per group

Table 4. Number of animals per group classified positive by the brucellosis indirect ELISA

Groups	D0	W1	W2	W3	W4	W5	W6	W7	W8
BA (5)°	0	0	0	1	1	2	3	3	3
BS (2)	0	0	2	2	2	2	2	2	2
YO9 (6)	0	3	6	6	6	6	6	6	6
YO3 (3)	0	0	0	0	0	0	0	0	0
FPSR (4)	4	4	3	3	3	2	3	3	3

( )°: number of animals per group

Table 5. Number of animals per group classified positive by the brucellosis competitive ELISA

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	0	0	0	1	0	0	0	2
BS (2)	0	0	2	1	0	0	0	0	0
YO9 (6)	0	0	2	2	3	1	1	1	2
YO3 (3)	0	0	0	0	0	0	0	0	0
FPSR (4)	0	0	0	0	0	0	0	0	0

( )°: number of animals per group

Table 6. Number of animals per group classified positive by the brucellosis Interferon-γ test

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	0	nd	2	1	nd	3	nd	2
BS (2)	0	0	nd	1	1	nd	2	nd	0
YO9 (6)	0	0	nd	nd	0	nd	1	nd	1
YO3 (3)	0	0	nd	nd	0	nd	3	nd	0
FPSR (4)	0	0	nd	nd	0	nd	0	nd	0

( )°: number of animals per group *nd: not done*

Table 7. Number of animals per group classified positive by the YOPs ELISA test

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	1	1	1	0	0	0	0	0	0
BS (2)	0	0	0	0	0	0	0	0	0
YO9 (6)	0	0	6	6	6	6	5	5	4
YO3 (3)	0	0	3	3	3	3	3	3	3
FPSR (4)	2	2	2	2	2	2	1	1	1

( )°: number of animals per group

Table 8. Number of animals shedding *Y. enterocolitica* O:3 (YO3) or *Y. enterocolitica* O:9 (YO9) in their faeces

Groups	*D0*	*W1*	*W2*	*W3*	*W4*	*W5*	*W6*	*W7*	*W8*
BA (5)°	0	0	0	nd	0	nd	nd	0	nd
BS (2)	0	0	0	nd	0	nd	nd	0	nd
YO9 (6)*	0	6	6	2	2	2	2	2	2
YO3 (3)**	0	2	3	2	2	2	2	2	2
FPSR (4)	1	1	1	1	1	1	1	1	1

( )°: number of animals per group *nd: not done*
: O:9 shedders* *: Y. enterocolitica O:3 shedders*

Field studies

The National Veterinary Laboratory tested 2 448 serum samples that were classified as positive in the provincial laboratories by the SAW (estimated individual prevalence rate: 1.8 per 1 000) by iELISA. The vast majority of these seroreactors were single reactors, i.e. one seropositive animal per herd (estimated serologically tested herd prevalence rate: 2.3 per cent). Only 151 sera were classified positive by the iELISA. This means that of the population selected by the SAW (and thus suspected to be infected), 95 per cent of the animals are classified as brucellosis negative. They do not present a serological profile considered indicative of *B. abortus* infection. Indeed, we could not produce experimental *B. abortus* infections, even using low infectious doses, that resulted in SAW positive results without also obtaining positive iELISA results as well.

The 151 animals that were classified positive by the SAW and the ELISA were all skin test negative, allowing the veterinary services to declare the country brucellosis free. Indeed, not a single case of brucellosis has been found in Belgium in 2001. These results are depicted in Figure 2.

DISCUSSION

Experimental infection

Our results show clearly that none of the different brucellosis serological tests used in this study is able to differentiate brucellosis from *Y. enterocolitica* O:9 infections, and, thus, confirms and supports previous reports (Kittelberger *et al*, 1995; Weynants *et al*, 1996; Garin *et al*, 1997; Saegerman *et al*, 1999).

The RB and the SAW can be used as screening tests to detect reactors in Group 1 as early as one week p. i. Both tests also detected all animals in Group 3 as early at two weeks p.i. However, the number of animals that can be detected diminishes with time and only one animal is still classified positive eight weeks p.i. These observations correlate with field observations where aspecific serological results waned with time so that more than 90 per cent of the seroreactors tested negative in the SAW after six weeks (Saegerman *et al*, 1997).

The CFT, which is used worldwide as a confirmatory test, detected all animals in Group 3. This suggests that the specificity of the CFT is questionable in animal populations where *Y. enterocolitica* is present. It is important to note that the CFT did not detect a persistently *B. abortus* infected animal. Such latent infections may account for the re-emergence of brucellosis in herds where a partial test-and-slaughter policy includes only CFT positive animals. Thus, it would be difficult and, hence, dangerous to designate an area as being freed of brucellosis based on negative CFT results (Plommet *et al*, 1973; Crawford *et al*, 1986).

The iELISA is the most sensitive test with all animals in Group 3 testing positive as early as two weeks p.i. and remaining positive until the end of the experiment. Therefore, in a testing strategy, the iELISA detects both brucellosis and infections due to *Y. enterocolitica* O:9 but can not discriminate between them. Thus, other tests are needed to determine the status of the animals. Competitive ELISA were reported as being able to eliminate cross-reactions problems, although competing antibodies had been observed after *Y. enterocolitica* O:9 infections (Nielsen *et al*, 1990). In our studies, the brucellosis competitive ELISA did not solve the problem,although the specificity seems to be better than that of the iELISA. However, because of its lack of sensitivity it did not allow us to detect one *B. abortus* biovar 1 persistently infected animal and to detect any of the *B. suis* biovar 2 infected animals that had been classified positive by the iELISA. Four MAbs against S-LPS have been reported with specificity to either the A epitope, the M epitope or the common epitopes cross-reacting (C/Y) or the non-crossreacting (C) S-LPS epitopes from *Y. enterocolitica* O: 9. Although the M epitope is absent on the O chain of *Y. enterocolitica* O: 9 (Caroff et al, 1984), all the animals of Group 3 are classified positive in this test. Competitive binding assays between different classes of anti-LPS monoclonal antibodies suggest that the different epitopes are probably overlapping structures (Weynants *et al*, 1996). If this is true, it will make the development of a competitive ELISA specific for brucellosis difficult unless the C epitope could be synthetized or mimicked and used alone (Weynants *et al*, 1996; Kittelberger *et al*, 1998).

The brucellosis IFN-γ test showed disappointing results under these experimental conditions and can therefore not be recommended as a test discriminating *Y. enterocolitica* O:9 infections from brucellosis. Better antigens are needed or a control antigen (like for the tuberculosis IFN-γ test) has to be defined in order to increase the specificity of this test.

Because antibody responses to proteins of brucellae were often delayed compared to the antibody response to smooth LPS and because antibody responses to proteins were found to be limited to animals that developed an active brucellosis infection (Letesson *et al*, 1997), assays based on the detection of antiprotein humoral responses were not included in this study.

The YOPs-ELISA is an accurate test to detect a *Y. enterocolitica* group infection (Table 7). In one *B. abortus* infected animal, antibodies against YOPs were detected showing that dual infections may occur and may interfere with differ-

ential serological testing. Thus, unfortunately, as YOPs based assays will not determine the infectious status of an animal regarding brucellosis, their usefullness will be limited to epidemiological studies (Kittelberger *et al*, 1995; Weynants *et al*, 1996).

In order to validate serological tests, results need to be correlated to the infectious status of an animal. The presence of anti-brucellae antibodies suggests exposure to the bacteria, but it does not necessarily mean that the animals have a current or active infection at the time of sampling. The presence of antibodies may be a result of past infections resulting in a 'self-limiting' disease, as suggested in this study from the results obtained from the two *B. abortus* biovar 1 infected animals and the two *B. suis* biovar 2 infected animals.

We chose to infect animals with a dose of *B. abortus* biovar 1 that did not induce high titres in brucellosis serological tests in order to mimic latent infections in a herd. The brucellosis bacteriology showed that it is virtually impossible to detect, beyond any doubt, all *B. abortus* infected animals by means of serology and, thus, the successful conclusion of the eradication program has been reached. Although all animals in a *B. abortus* infected herd are not infected, total depopulation seems to be the best option to avoid re-emergence of the infection.

The most specific test is the brucellosis skin test. These experimental data provide additional support for the implementation of the brucellosis skin test as a recommended herd test for brucellosis in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Anon., 2000) and as an official test in the EU (amendment of the Directive 64/432), particularly when monitoring is made difficult due to aspecific brucellosis serological reactions.

Testing strategy applied during the 2001 Belgian eradication campaign

When detecting rare events, like cases of brucellosis at the end of the eradication program, the emphasis is no longer on the intrinsic values of a test, but rather on its specificity or positive predictive value as this relates to the clinical utility of the result (Gardner *et al*, 1996; Pouillot *et al*, 1997; Saegerman *et al*, 1997; Saegerman *et al*, 1999). In a screening program, the first line test should be chosen to identify and select a population 'at risk'. The SAW or RB screening tests can be used for this purpose. The proportion of serological cross-reactions in the brucellosis serological tests induced by cross-reactive bacteria like *Y. enterocolitica* O:9, which are almost indistinguishable from true brucellosis serological reactions (Weynants *et al*, 1995, Saegerman *et al*, 1999), is increasing as the end of the program nears. In some regions of Belgium, the animal prevalence rate varied from 1 to 10 per 10 000, and the herd prevalence rate from 2 to 12 per 100, during the nineties (Saegerman *et al*, 1997). Therefore, other tests or combinations of tests have to be used, besides a sound proficient epidemiological inquiry in order to identify risk factors like age of the animal, herd size as well as a seasonal effect (Saegerman *et al*, 1997; Pouillot *et al*, 1998). Criteria other than positive serology are, therefore, important in an eradication program.

If brucellosis is present in wildlife, the concern of the livestock industry is the prevention of the re-introduction of the infection in livestock. This is particularly true in regions or states that are 'brucellosis officially free' because of the implications for pre-movement testing for domestic animals (Godfroid, 2002). In Belgium, the fact that brucellosis is present in wild boars does not seem to impact the bovine brucellosis eradication program because, although *B. suis* biovar 2 has been shown to induce a 'self-limiting disease' in experimentally infected animals, these animals were classified as positive by the skin test. No skin test positive reactions were seen in the 151 animals classified SAW+ and iELISA+.

The aim of an eradication program is not a 'zero seropositivity' situation, but, rather, it is the absence of infection even if seropositivity is encountered. Criteria to declare a country or a region 'officially free from brucellosis' have been laid down in international regulations, like in EU Directives or by the OIE. However, the actual epidemiological situation is not taken into consideration. The tests are applied without regard to the true incidence and prevalence rates. Hence, the positive predictive value of tests varies according to the progress of the eradication program. This leads to a contradiction: a country or a region can be looked upon as being 'biologically' free from *B. abortus* infection of cattle but not 'administratively' free (Dufey, 1992). The implementation of the strategy reviewed here shows 1) that it is possible by a combination of tests and epidemiological inquiries to substantiate the eradication of brucellosis, even in the presence of aspecific serological reactions and 2) that factors, in addition to test results, are important to guarantee the success of an eradication program.

ACKNOWLEDGEMENTS

The experimental part of this work was supported by a grant of the European Union (contract n° FAIR CT 95-0134).

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THE DISEASE RISKS OF EXPORTING BOVINE, OVINE AND CAPRINE SEMEN FROM SOUTH AFRICA TO AUSTRALIA

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SUMMARY

Biosecurity Australia is currently conducting an import risk analysis (IRA) on importing bovine, ovine and caprine semen from South Africa. This IRA may provide South Africa with an opportunity to develop export markets for its ruminant semen outside the African continent. This paper describes the progress to date with the risk assessment of several important diseases.

INTRODUCTION

In recent years, Australia has received access requests for cattle, sheep and goat semen from the Republic of South Africa (RSA). As there are no existing quarantine conditions under which ruminant semen from RSA can directly enter Australia, Biosecurity Australia (BA) is undertaking an IRA to evaluate the risks of entry, establishment and spread of pests and diseases and their potential impacts.

Importing animals and animal products involves a degree of disease risk to importing countries. An IRA provides importing countries with an objective and defensible method of assessing these disease risks. The analysis process used by BA is transparent so that the exporting country can understand why import conditions are imposed. Transparency is also essential because data are often uncertain or incomplete and, without full documentation, the distinction between facts and the analyst's value judgements may blur.

BA has published an *Import Risk Analysis Process Handbook* that provides details on the IRA process. The process is being reviewed to improve consultation with stakeholders and to affirm the scientific basis of the process. The IRA process conforms with OIE International Animal Health Code (2002) recommendations: 'An import risk analysis begins with a description of the commodity proposed for import and the likely annual quantity of trade. It must be recognised that whilst an accurate estimate of the anticipated quantity of trade is desirable to incorporate into the risk estimate, it may not be readily available, particularly where such trade is new.' Hazard identification is an essential step which must be conducted before the risk assessment. The risk assessment process consists of four interrelated steps. These steps clarify the stages of the risk assessment, describing them in terms of the events necessary for the identified potential risk(s) to occur, and facilitate understanding and evaluation of the outputs. The product is the risk assessment report which is used in risk communication and risk management. (Figure 1)

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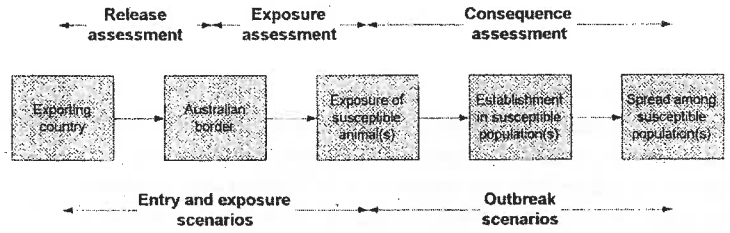
Figure 1. Likelihood components of risk assessment



The four interrelated steps are:

1. Release assessment, which consists of describing the biological pathway(s) necessary for an importation activity to 'release' (that is, introduce) pathogenic agents into a particular environment, and estimating the probability of that complete process occurring, either qualitatively or quantitatively. The release assessment describes the probability of the 'release' of each of the potential hazards (the pathogenic agents) under each specified set of conditions with respect to amounts and timing, and how these might change as a result of various actions, events or measures. (Figure 2)

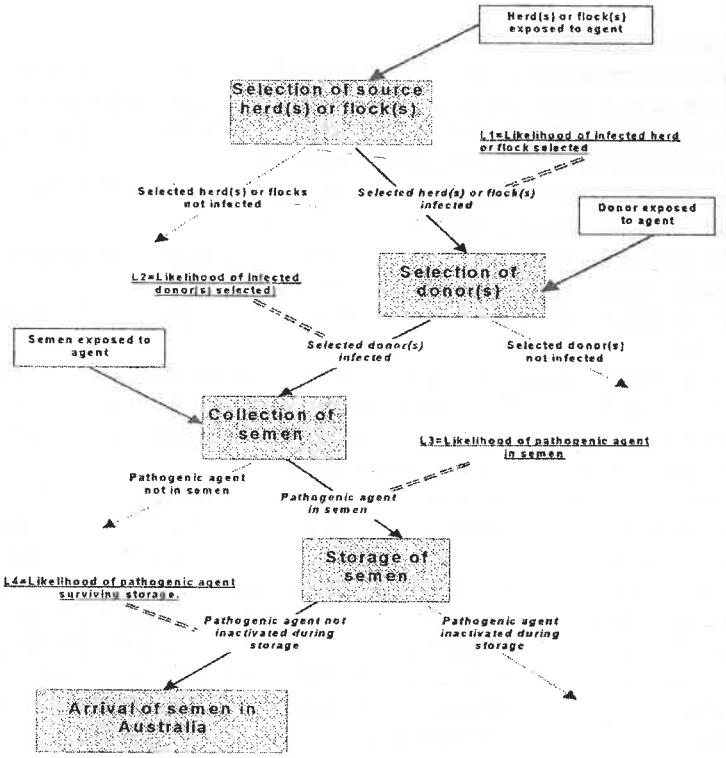
2. Exposure assessment, which consists of describing the biological pathway(s) necessary for exposure of animals and humans in the importing country to the hazards (in this case the pathogenic agents) released from a given risk source, and estimating the probability of the exposure(s) occurring, either qualitatively or quantitatively.

3. Consequence assessment, which consists of describing the relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process must exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. This estimate may be either qualitative or quantitative.

4. Risk estimation, which consists of integrating the results from the release assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset. Thus risk estimation takes into account the whole of the risk pathway from hazard identified to unwanted outcome.

This paper describes the hazards identified to date and some problems being encountered in analysing the risks associated with these pathogenic agents occurring in semen and subsequently infecting the inseminated animals and spreading the infection.

Figure 2. Release scenario for the importation of semen



METHODS

The import risk analysis approach

The first step in the IRA process for ruminant semen from RSA was for BA to consult with the stakeholders, proposing the IRA commence and be developed in-house. The stakeholders who responded to BA's proposals agreed with this approach. However, an opportunity was provided for other stakeholders to appeal against this approach. No appeals were received.

The second step was to publish a technical issues paper, which included Australia's current policy for bovine, ovine and caprine semen and outlined the results of hazard identification.

The third step will be to publish the draft IRA which will contain the methods for, and the results of, risk assessment and risk management and provide a preliminary position on the importation of bovine, ovine and caprine semen from RSA. After receiving and evaluating comments on the draft IRA by the stakeholders, the final IRA, which will include the same elements with any necessary

revisions, and a description of quarantine conditions for bovine, ovine and caprine semen from RSA will be published.

Hazards identified

Hazard identification is described in the OIE Code as a classification step, with the purpose of identifying pathogenic agents (or clearly identified strains of pathogenic agents) that could be associated with the importation of a commodity. Agents thus classified are termed 'potential hazards'.

The OIE Code states that in order to be identified as a potential hazard, a pathogenic agent should comply with all of the following criteria:

- The pathogenic agent should be appropriate to the animal species to be imported, or from which the commodity is derived.
- The pathogenic agent could produce adverse consequences in the importing country.
- The pathogenic agent may be present in the exporting country[1].
- The pathogenic agent should not be present in the importing country. If present, the pathogenic agent should be associated with a notifiable disease, or should be subject to control or eradication measures[2].

Hazard identification was initiated by generating a comprehensive list of disease agents likely to be relevant to the importation of bovine, ovine and caprine semen from the RSA. The list included those disease agents associated with OIE List A and B diseases and known to affect cattle, sheep and goats, and any other agents considered relevant to bovine, ovine and caprine semen from the RSA. The list was subsequently refined by applying to each disease agent, the four criteria stated above. If reasons for the inclusion/exclusion or particular pathogenic agents were not clear-cut, these agents were retained on the list and examined in the formal risk assessment.

Table 1. Hazard identification

Preliminary identification	*Retained for further consideration in IRA?*
OIE List A diseases	
Foot and mouth disease (Foot and mouth disease virus)	Yes
Vesicular stomatitis (Vesicular stomatitis virus)	No
Rinderpest (Rinderpest virus)	No
Pestes des petits ruminants (Pestes des petits ruminants virus)	No
Contagious bovine pleuropneumonia (*Mycoplasma mycoides mycoides*)	No
Lumpy skin disease (Lumpy skin disease virus)	Yes
Rift Valley fever (Rift Valley fever virus)	Yes
Bluetongue (Bluetongue virus)	Yes
Capripox (Capripox virus)	No

[1]The OIE Code states that ' ... *the evaluation of the veterinary services, surveillance and control programs and zoning and regionalisation systems are important inputs for assessing the likelihood of hazards being present in the animal population of the importing country ...*'

[2]In this context, 'control or eradication measures' are taken to mean a compulsory control or eradication program.

Preliminary identification	*Retained for further consideration in IRA?*
OIE List B diseases	
Anthrax (*Anthracis bacillus*)	No
Aujeszky's disease (Aujeszky's disease virus)	No
Hydatidosis (*Echinococcus granulosus and E multilocularis*)	No
Heartwater (*Cowdria ruminantium*)	Yes
Leptospirosis (*Leptospira* spp.)	No
Q-fever (*Coxiella burnetii*)	No
Rabies (Rabies virus)	No
Johne's disease (*Mycobacterium (avium* subsp.) *paratuberculosis*)	Yes
Screw-worm fly (*Cochliomyia hominovorax*)	No
Anaplasmosis (*Anaplasma marginale*)	No
Bovine babesiosis (*Babesia* spp)	No
Bovine brucellosis (*Brucella abortus*)	Yes
Campylobacteriosis (*Campylobacter fetus* subsp *fetus*)	No
Bovine tuberculosis (*Mycobacterium bovis*)	Yes
Bovine cysticercosis (*Cysticercus bovis*)	No
Dermatophilosis (*Dermatophilus congolensis*)	No
Enzootic bovine leucosis (Bovine leukemia virus)	Yes
Haemorrhagic septicaemia (*Pasteurella multocida* serotypes B:2 & E:2)	Yes
Infectious bovine rhinotracheitis/ Infectious pustular vulvovaginitis (Bovine herpesvirus 1)	Yes
Theileriosis (*Theileria* spp - *T. parva, T. annulata, T. mutans*)	Yes
Trichomoniasis (*Tritrichomonas foetus*)	No
Trypanosomiasis (*Trypanosoma* spp)	Yes
Bovine malignant catarrhal fever (Bovine malignant catarrhal fever virus)	No
Bovine spongiform encephalitis	No
Ovine brucellosis (*Brucella ovis*)	Yes
Brucellosis (*Brucella melitensis*)	Yes
Caprine arthritis/encephalitis (Caprine arthritis/encephalitis virus)	No
Contagious agalactia (*Mycoplasma agalactiae*)	No
Contagious caprine pleuropneumonia (*Mycoplasma capricolum subsp. capripneumoniae*)	No
Enzootic abortion of ewes (*Chlamydia psittaci*)	Yes
Jaagsiekte (Jaagsiekte virus)	Yes
Nairobi sheep disease (Bunyavirus)	No
Salmonellosis (*Salmonella abortus ovis*)	No
Scrapie	No
Maedi-visna (Maedi-visna virus)	Yes
Other diseases/disease agents	
Louping-ill and other tick borne encephalitides (Antigenically related flaviviruses	No
Bovine viral diarrhoea/Mucosal disease - BVDV, Border disease BDV (Pestivirus)	Yes
Anaplasmosis (*Anaplasma* spp of sheep and goats)	No
Borna disease (Borna disease virus)	No
Wesselsbron disease (Wesselsbron disease virus)	Yes
Sheep scab (*Psoroptes ovis*)	No
Crimean-Congo haemorrhagic fever (Crimean-Congo haemorrhagic fever virus)	Yes
Besnoitiosis (*Besnoitia* spp)	Yes
West Nile disease (West Nile virus)	No

At the time of preparing this paper, comments from stakeholders on the Technical Issues Paper have not been collated and the hazards list may be modified, depending on the comments and suggestions received by the stakeholders.

Preliminary risk assessment

The approach being adopted by BA in-house ruminants risk analysis team is based on the principles of evidence-based medicine, which is the conscientious, explicit and judicious use of current best evidence in assessing risks. As quantitative data is not available in the majority of cases, the risks are being assessed qualitatively. Some work has already been done in evaluating the risks but the limited information on the likelihood of semen being infected with many diseases not found in Australia and on the risks of infected semen infecting inseminated females and sreading makes it very difficult to scientifically estimate the risks these diseases pose to Australia. Examples include:

Foot and mouth disease virus (FMDv) There is experimental and epidemiological evidence of FMDv in semen of cattle and buffalo. Epidemiologic investigations suggest that sexual transmission of FMDv from mature carrier Cape buffalo bulls to domestic cows can occur.[1] Experimental insemination of heifers with semen infected with two strains of FMDv has resulted in infection in these heifers.[3] While it is known that FMDv can be detected in semen collected from boars infected with FMDv[9], little is known about whether FMDv can also infect ovine and caprine semen.

Lumpy skin disease virus (LSDv) Many texts have stated that LSDv can be detected in semen of infected bulls. However the only evidence of this occurring was a report of unpublished observations by Alexandra and Weiss in 1959.[18] There is no published information as to whether infected semen can infect inseminated cows.

Rift Valley fever virus (RVFv) Fortunately RVF is a rare occurrence in RSA. While RVF is a mosquito-borne viral disease, RVFv can be excreted in milk, saliva[7] and nasal discharges during the viraemic phase in infected animals. There are no reports of RVFv in semen nor are there reports of sexual transmission. A laboratory study has demonstrated the presence of competent mosquito vectors for RVFv in Australia.[16]

Bluetongue virus (BTv) While BTv occurs in Australia, clinical bluetongue does not. Of the 1 400 species of *Culicoides* worldwide, less than 20 are known vectors of BTv. *C. imicola* and *C. bolitinos*, known vectors of BTv in RSA, do not occur in Australia. Known Australian vectors include *C. brevitarsus*, *C. wadai*, *C. fulvis* and *C. actoni*. RSA and Australia have different strains and serotypes of BTv. It is not known whether Australia *Culicoides* can act as suitable vectors of South African strains. The evidence for sexual transmission of BTv is equivocal. In Australia, mature bulls infected with laboratory cultured BTv can excrete the virus in their semen but the virus could not be identified in the semen of young bulls experimentally infected with either laboratory cultured or wild-type unadapted virus or in older bulls infected with wild-type

unadapted virus.[10] On the other hand, American reports suggest natural sexual transmission of BTv can occur.[2;8]

Cowdria ruminantium (Cr) While heartwater is a tick-borne disease, occurring only where its vectors, some ten members of the *Amblyomma* genus, are present, vertical transmission of heartwater can occur, either by intra-uterine transmission of Cr or by colostral cells from infected animals.[4;17] There is no information on Cr in semen or on possible sexual transmission. Relatively little is known about the epidemiology of the disease. Five Amblyomma species have been reported in Australia, none of which have been detected on ruminants.[6]

Theileria parva lawrencei (Tpl) Corridor disease (CD) occurs sporadically throughout southern and eastern Africa wherever there is contact between cattle and infected African buffalo, *Syncerus caffer*, in the presence of suitable tick vectors, namely *Rhipicephalus appendiculatus*, *R. zambeziensis* or *R. duttoni*. CD is recognised as a self-limiting disease in cattle. Initial findings suggest that in the absence of suitable tick vectors and African buffaloes Tpl is not likely to spread from an infected animal in Australia.

Trypanosoma spp The distribution of *T. brucei* and *T. congolense* is restricted to the tsetse infected tracts of Africa and initial findings suggest these parasites cannot establish in Australia. However, *T. vivax* has shown remarkable ability to adapt away from the tsetse fly hosts, having adapted to parts of Africa where tsetse fly has been eradicated and to parts of South America where it is spread by tabanid flies, vampire bats and possibly by needles used in mass vaccination of stock against FMD. While *T. vivax* can cause genital lesions and infertility in rams, bucks and bulls, irrespective of the severity of the general clinical signs, there is no report of the parasite in semen,[11;13-15]or sexual transmission.

Brucella ovis Testing rams for ovine brucellosis is standard procedure in most artificial breeding centres. Infection in rams is lifelong whereas in ewes it is usually shortlived, lasting one or two heat cycles, although persistent infection has been reported.[5] Transmission is by passive venereal contact with the ewes during the mating season or by direct ram-to-ram transfer through oral or mucosal routes. Mating of non-pregnant ewes with infected rams usually results in vaginitis and cervicitis in a small proportion of ewes. Infection in such ewes persists for 24 to 48 hours but may recur at the following oestrus if pregnancy is not established or at the first oestrus after parturition if pregnancy is established, and may result in increased proportions of ewes returning to service. Ewes inseminated with infected semen, which has already been considerably diluted during processing, are less likely to become infected and, if infected, are not considered to be a source of infection. This raises the question as to whether testing of donor rams for *B. ovis* is necessary as semen needs to pass the quality test before being processed for artifical insemination. However, the IRA will also analyse the risks of using of *B. melitensis* Rev 1 vaccine for control of *B. ovis* in RSA

Chlamydia psittaci serotype 1 (or Chlamydophila abortus) Rams and bulls inoculated parentally can excrete chlamydiae in the semen near the end of the

bacteraemic phase and for up to 29 days after. Most information on infection in ewes relates to infection occurring during gestation and not at mating or artifical insemination. There is one report of abortion or birth of weak lambs occurring in ewes experimentally inoculated intravaginally prior to breeding.[12] However infective doses in experiments are usually far in excess of that in semen, processed or otherwise. Hence it is difficult to evaluate the risk of using infected semen in ewes during oestrus.

Information is lacking for several other pathogenic agents, including *Brucella melitensis*, jaagsiekte virus and maedi-visna virus. The above examples serve to highlight lack of information of the disease risks posed by ruminant semen.

CONCLUSION

Undertaking an IRA to analyse the disease risks of importing bovine, ovine and caprine semen from South Africa, to evaluate risk management options and to develop import conditions, is not a straightforward task. It requires considerable scientific research and extensive consultation with stakeholders and with scientific experts having practical experience with the disease. Most of the pathogenic agents identified as hazards have not been reported in Australia and assistance from South African experts will be required during the analysis.

Those who wish to follow the progress of the IRA or who wish to register as stakeholders are advised to look at BA's website (http://www.affa.gov.au/biosecurityaustralia).

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MOLECULAR CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES O, SAT-1, AND SAT-2 IN WEST AFRICA

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SUMMARY

Foot-and-mouth disease (FMD) virus is a highly contagious viral disease affecting cloven-hoofed animals. It has a positive sense, single stranded RNA genome and belongs to the genus Aphthovirus within the family Picornaviridae. Seven serotypes (A, O, C, SAT-1, SAT-2, SAT-3 and Asia1) of FMD virus exist worldwide and four (A, O, SAT-1 and SAT-2) have been isolated from West Africa. Although the epidemiology has been well studied in other parts of the world little is known about the epidemiology of the disease in West Africa.

In order to study the molecular epidemiology of FMD in West Africa, several FMD viruses (serotypes A, O, SAT-1 and SAT-2) causing outbreaks in this region between 1974 and 1999 were obtained from the World Reference Laboratory at Pirbright (UK). The genome region encoding the main antigenic capsid protein, VP1, was amplified by using the polymerase chain reaction (PCR) and nucleotide sequences were determined following agarose gel electrophoresis and purification.

Gene trees were constructed using partial and complete nucleotide sequences of the VP1 gene of viruses from West Africa. Additional strains from East, Central and Southern Africa were incorporated in the study for comparative purposes. Results indicate the presence of three distinct evolutionary lineages for SAT-2 viruses from West Africa and at least two different genotypes within serotypes O and SAT-1. For all serotypes, viruses clustered according to year of isolation rather than geographical origin. When viruses from West African were compared with viruses representative of other African regions, it was found that the strains from West Africa were consistently genetically distinct from viruses occurring elsewhere on the continent, for all four FMD serotypes. This genetic distinctiveness indicates that control of the disease through vaccination requires the incorporation of regionally unique vaccine strains in order to be effective. The results further show that transboundary transmissions have occurred on numerous occasions in West Africa.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting

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over 40 different species of domestic and wild cloven-hoofed. Although mortality is rare in adults, morbidity can approach 100 per cent. Economically, it is the most devastating disease of livestock. The cost of the recent UK epidemic is expected to exceed US$29 billion (Samuel and Knowles, 2001).

The disease is caused by a single-stranded, positive sense RNA virus, of the Aphthovirus genus, within the family Picornaviridae. FMD virus occurs as seven immunologically distinct serotypes, six of which occur on the African continent (Brooksby, 1972). Between 1974 and 1993, four different FMD serotypes were recorded in West Africa, namely, types A, O, SAT-1 and SAT-2. Types A and O, two of the so-called European serotypes due to their extensive historical distribution on that continent, primarily occur north of the equator in Africa and are not associated with wildlife. In contrast, SAT-1 and SAT-2 are widely distributed throughout sub-Saharan Africa and have a close link to wildlife, particularly African buffalo (*Syncerus caffer*) in southern and eastern Africa. The role of wildlife in the epidemiology of the latter serotypes in West Africa is not known, but is presumed to be negligible given the limited number of wildlife present in this region.

With the exception of type A (Knowles *et al*, 1998), the epidemiology of FMD in West Africa remains obscure. The aim of this study is to use molecular methods to obtain insight into the origin and course of epizootics caused by types O, SAT-1 and SAT-2 viruses in West Africa. In order to achieve this, the immuno-dominant and genetically variable VP1 surface-exposed protein was targeted for genetic characterisation.

MATERIALS AND METHODS

Genomic amplification and nucleotide sequencing

Viral nucleic acid was extracted by a guanidine-silica method (Boom *et al*, 1990) and RNA was reverse transcribed with a universal antisense primer 5'-GAA GGG CCC AGG GTT GGA CTC-3' (Beck and Strohmaier, 1987), and 10 U of AMV-RT (Promega) as previously described (Bastos, 1998). Genomic amplification was initially attempted with a variety of published primers (Bastos 1998; Rodríguez *et al*, 1995), but due to the poor success rate of viruses of the endemic serotypes, new primers had to be designed to ensure amplification of SAT-1 and SAT-2 viruses from West Africa. These novel West African-specific primers were determined on the basis of nucleotide sequences generated for the low proportion of amplification attempts that were successful. Ultimately, a novel sense strand primer was combined with the universal antisense primer (Beck and Strohmaier, 1987) and used for genomic amplification. Products of 581 bp, 750 bp, and 518 bp were obtained in this way for serotypes O, SAT-1 and SAT-2, respectively. DNA bands were recovered after agarose gel electrophoresis and purified according to supplier specifications, following which nucleotide sequences were generated manually (Bastos, 1998) or by automated cycle sequencing.

Data analysis

Nucleotide sequences were aligned using DAPSA (Harley, 2001) and exported in Clustal format to a word processing package where formatting of data in MEGA format ensued prior to export as a text file. Three independent datasets were analysed, namely, a type O dataset, 495 nt, long corresponding to amino acid positions 49-213 of VP1, a SAT-1 dataset 666 nt in length encoding the entire VP1 gene and a SAT-2 dataset 480 nt long corresponding to amino acid positions 48-215 of the VP1. Neighbor-joining (NJ) and UPGMA methods included in MEGA 2 (Kumar *et al*, 2002) were used to infer a phylogeny, using both uncorrected (p-distances) as well as corrected distances. Data were resampled between 1 000 and 10 000 times in order to determine the statistical significance of nodes. A phylogeny was also inferred for each of the datasets using maximum parsimony (MP).

RESULTS

Gene trees of similar topology were consistently obtained irrespective of the method of analysis used, for each of the three West African serotypes. This indicates that the inferred phylogenies are reliable (Kim, 1993). For each of the serotypes, epizootics were shared by neighbouring countries indicating that transboundary virus transmission is a regular occurrence in West Africa (summarized in Table 1).

Genetic relationships of type O FMD viruses

Two distinct West African genotypes were distinguished:

(1) Niger (1988)

(2) Burkina Faso (1992) and Ghana (1993)

Viruses from Burkina Faso 1992 and Ghana 1993 although derived from outbreaks occurring in different years and in different countries, had >98 per cent sequence identity, indicating that they are part of the same epizootic and have a common origin.

Genetic relationships of type SAT-2 FMD viruses

Genetic characterization of SAT-2 viruses isolated from 1974-1991 in West Africa revealed the presence of three major evolutionary lineages, i.e. virus lineages that differ from each other by more than 25 per cent on nucleotide sequence level. Within West Africa, three genotypes were observed:

- (A) Ghana and Ivory Coast (1990), Ghana and Mali (1991);
- (B) Ghana, Nigeria, Liberia & Ivory Coast (1974), Senegal and Nigeria (1975);
- (C) Gambia and Senegal (1979), Senegal (1983).

Incorporation in the analysis of SAT-2 viruses from East and Central Africa revealed that SAT-2 type viruses from West Africa are genetically distinct from those occurring elsewhere on the continent.

A circulation period of up to four years (1979-1983) was observed for the

Table 1. Summary of trans-boundary FMD epizootics in West Africa

Isolate name	*Year of outbreak*	*Country of origin*	*Serotype*	*Sequence identity*
BKF/1/92	1992	Burkina Faso		
BKF/2/92	1992	Burkina Faso		
BKF/3/92	1992	Burkina Faso		
GHA/9/93	1993	Ghana	Type O	> 98 %
GHA/6/93	1993	Ghana		
GHA/7/93	1993	Ghana		
GHA/5/93	1993	Ghana		
LBR/1/74	1974	Liberia		
IVY/9/74	1974	Ivory Coast		
GHA/10/74	1974	Ghana		
GHA/14/74	1974	Ghana		
NIG/35/74	1974	Nigeria	SAT-2	> 96 %
NIG/1/74	1974	Nigeria		
SEN/1/75	1975	Senegal		
SEN/5/75	1975	Senegal		
NIG/1/75	1975	Nigeria		
NIG/2/75	1975	Nigeria		
GAM/8/79	1979	Gambia		
GAM/9/79	1979	Gambia		
SEN/7/79	1979	Senegal		
SEN/8/79	1979	Senegal	SAT-2	> 97 %
SEN/3/83	1983	Senegal		
SEN/7/83	1983	Senegal		
SEN/1/83	1983	Senegal		
SEN/5/83	1983	Senegal		
IVY/2/90	1990	Ivory Coast		
IVY/7/90	1990	Ivory Coast		
GHA/2/90	1990	Ghana		
GHA/8/90	1990	Ghana		
MAI/1/91	1991	Mali		
MAI/3/91	1991	Mali	SAT-2	> 97 %
MAI/2/91	1991	Mali		
MAI//5/91	1991	Mali		
MAI/6/91	1991	Mali		
MAI/7/91	1991	Mali		
NIG/24/75	1975	Nigeria		
NIG/25/75	1975	Nigeria		
NIG/15/75	1975	Nigeria		
NIG/1/75	1975	Nigeria		
NGR/1/76	1976	Niger	SAT-1	>97
NGR/2/76	1976	Niger		
NGR/4/76	1976	Niger		
NGR/5/76	1976	Niger		

genotype C field isolate in Senegal.

Genetic relationships of type SAT-1 FMD viruses

Analysis of complete VP1 gene sequences of SAT-1 viruses from West Africa indicated the presence of two SAT-1 lineages:

- Lineage I: isolates from Niger and Nigeria (1975-1976);
- Lineage II: isolates from Nigeria (1979-1981).

The complete gene sequences further revealed the presence of two codon deletions at amino acid positions 81 and 148 lineage I viruses, whilst all lineage II isolates had only one codon deletion at amino acid 81 position. Comparison of West African SAT-1 viruses with those from southern and central Africa indicate that SAT-1 viruses from West Africa are unique to this region.

DISCUSSION

Results from molecular epidemiological studies conducted on serotype O, SAT-1 and SAT-2 viruses from West Africa correspond well with the findings of a similar type A study (Knowles *et al*, 1998), where West African viruses were shown to be genetically and geographically distinct from other viruses. These results have implications for the control of the disease through vaccination as genetic diversity corresponds well with antigenic variation, thus, viruses that are genetically unrelated also tend to have a poor antigenic relationship (Esterhusen 1994; Hunter *et al*, 1996). As present vaccines do not incorporate viruses of West African origin, custom-made vaccines will need to be developed for use in West Africa. The results of this study also indicate that animal movement is not restricted in West Africa as numerous West African countries are affected by a single outbreak strain. It is clear from these findings that control of the disease must incorporate control of animal movement in order to be effective.

ACKNOWLEDGEMENTS

We would like to thank N. Ferris of the Animal Health Institute, Pirbright, UK for supplying the isolates used in this study and Intervet International, The Netherlands for financial assistance. Oumou Sangare is supported by a Ph.D. scholarship from Winrock International.

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THE EPIDEMIOLOGY OF THE 2000 –2001 FOOT-AND-MOUTH DISEASE OUTBREAK IN NKOMAZI, MPUMALANGA, SOUTH AFRICA

B J A DU PLESSIS*

SUMMARY

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

From 1992 to 1997, the buffer zone, where vaccination is done, was gradually reduced in extent. Starting in 1998, the veterinary fence along the KNP boundary was upgraded to an electrified game fence. Before completion of this project, devastating floods in February 2000 damaged the upgraded and old sections of the fence, causing a drastic increase in recorded incidents of stray buffalo out of the KNP. Subsequent contact between buffalo and cattle lead to transmission of virus. Infection amongst cattle eventually spread through movements of cattle to other districts in Mpumalanga, as well as to Swaziland. Epidemiological factors that played a role in the spread and detection of the disease are assessed.

Two control campaigns were launched in November 2000 in Mpumalanga – one in respect of the disease in a cattle and sheep feedlot near Middelburg and the other in the Nkomazi area. By the end of March 2001 the situation in both instances was completely under control. Epidemiological factors considered in the control of the disease are discussed briefly.

Clinical and serological surveillance was done to determine the extent and the routes of spread of the disease as well as to evaluate vaccination response. Findings are discussed.

Evaluation of the entire experience was done and findings and recommendations are being considered in alterations to control measures.

INTRODUCTION

The Ehlanzeni / Lowveld Region of the Mpumalanga Province of South Africa borders onto the Kruger National Park (KNP), where Cape buffalo (*Syncerus caffer*) populations are persistently infected with foot-and-mouth disease (FMD) virus serotypes SAT-1, SAT-2 and SAT-3. Control measures such as movement control, vaccination and surveillance have been enforced since the 1930's in

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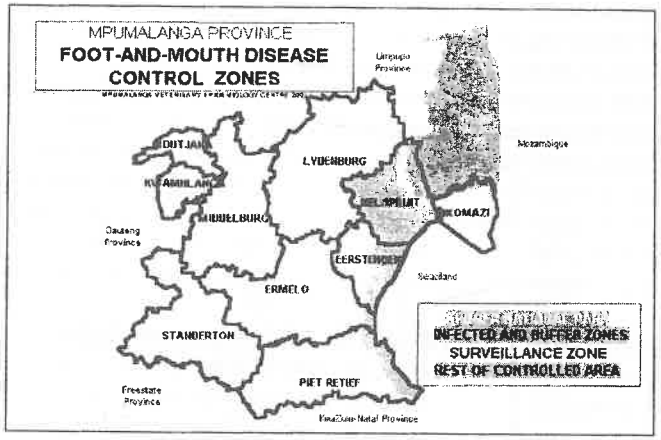
farming areas adjacent to the KNP, in order to prevent transmission of the virus to susceptible animals.

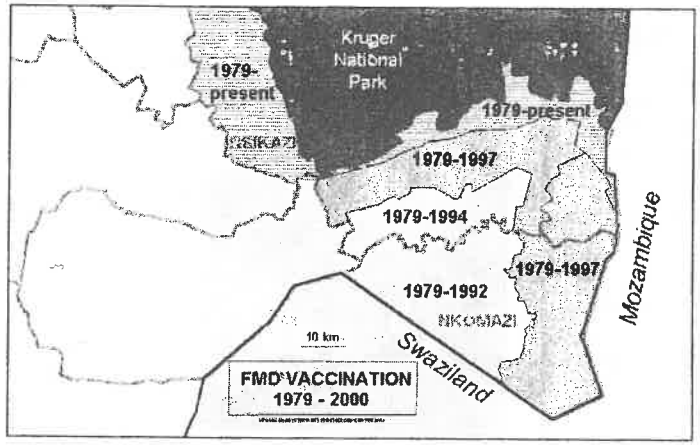
Figure 1. Map of Mpumalanga Province showing State Veterinary areas and FMD control zones



The declared controlled area is divided into infected, buffer and surveillance zones, where movement control, vaccination and inspection of susceptible animals are practiced to varying degrees, according to the risk of spread of the disease.

From 1992 to 1997, the buffer zone, where vaccination is done, was gradually reduced in extent, due to cost, promotion of marketing and a perceived decreased

Figure 2. Map of the Mpumalanga Lowveld showing the extent of vaccination against FMD from 1979 to 2000 (before the outbreaks)



risk. Since 1998 only cattle were vaccinated every six months in a narrow buffer zone around infected buffalo populations.

The reduction in the vaccination zone lead to cattle in previously vaccinated areas with varying immunity and protection levels against FMD. In 1997 this aspect was evaluated serologically. It was found that 80 per cent of cattle that were vaccinated five years earlier for the last time, were still sero-positive for SAT-1. This finding made sero-surveillance in previously vaccinated areas valueless as far as detecting infection was concerned.

Livestock inspections are done regularly at seven, 14 and 28-day intervals. Serological surveillance is only done occasionally due to vaccination and high cost.

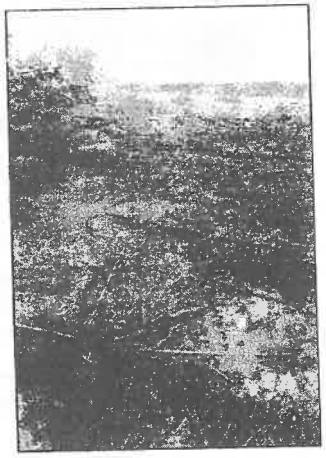
Movement control consists of a veterinary fence along the southern and western boundary of the KNP and a movement permit system, including inspections, quarantine, treatment and serology in respect of movements of susceptible animals and their products. Incidents of stray buffalo out of the KNP are regularly reported, closely monitored and swiftly acted upon by the veterinary directorate.

Contributing factors to FMD outbreaks, detection and spread

An increase in the KNP buffalo population, closing of artificial water points in the KNP and expanding sugarcane and fruit farming lead to an increase in buffalo presence along the Crocodile River, which forms the southern boundary of the KNP.

Upgrading of the 40 year-old veterinary fence along the KNP boundary to an electrified game fence in order to improve movement control, started in 1998. Before completion of this project, devastating floods in February 2000 damaged the upgraded and old sections of the fence. The fence damage report revealed that 14 per cent of the fence was completely gone, 36 per cent was flattened and

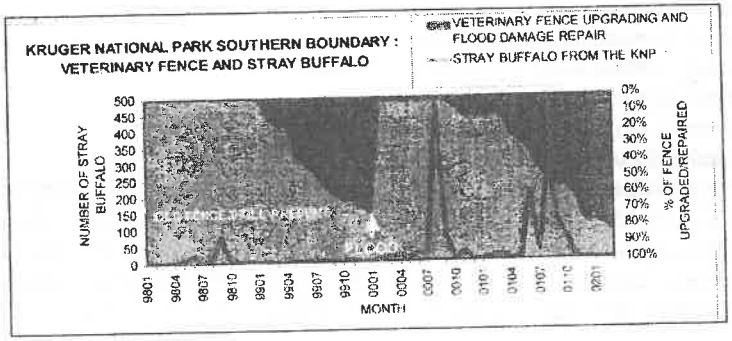
Figure 3. The flood of February 2000 caused severe damage to the veterinary fence



the remaining 50 per cent was still upright, but mostly clogged with debris and flotsam, eroded underneath, silted up, loosened and electrically non-functional.

Soon after the river levels had subsided, the damaged fence caused a drastic increase in recorded incidents of stray buffalo out of the KNP. Normally the incidence of stray buffalo across the KNP southern boundary peaks in late winter due to concentration of buffalo along the river because of deteriorating grazing conditions and scarcity of water, and due to easier crossing. In the winter of 2000, this peak reached an all time high.

Figure 4. The relationship between the veterinary fence and stray buffalo from the KNP



Subsequently two observed and one suspect incident of contact between stray buffalo and cattle lead to transmission of virus (FMD SAT-1). Two of these outbreaks amongst cattle eventually spread through contact between and movements of cattle to other districts (White River and Middelburg) in Mpumalanga, as well as to Swaziland.

Figure 5. Summary of available data on 3 incidents of buffalo / cattle contact that resulted in FMD SAT-1 virus transmission

Date of contact	*Farm name*	*Number and status of cattle*	*Buffalo involved*	*Fate of buffalo*	*Further spread*
2000-04-05 (observed)	Elsjan	56 routinely vaccinated	5 females and 1 male	All destroyed	None
2000-04-18 (observed)	Rooigras	114 (part of 1 528 of same owner) vaccinated until Oct. 1997	1 female	All destroyed	Yes
After 2000-08-08 (suspect)	Castilhopolis	583 vaccinated until Oct. 1997	2 females	Not traced	Yes

Various factors contributed in varying extent to the fact that the disease was not detected initially:

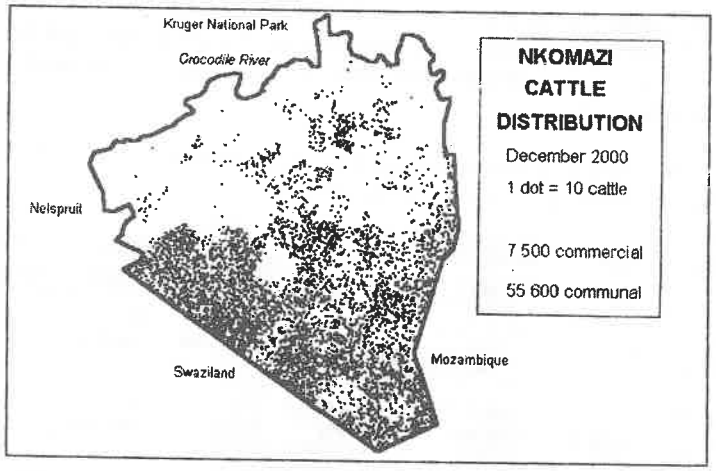
- Many cattle were still immune to a certain extent due to previous vaccination;
- The virus seemed to be less virulent (mild lesions were seen);
- Some cattle were absent during inspections;
- Permitted cattle movements within one zone do not require quarantine inspections;
- Diseased cattle were moved illegally (without a permit) from one infected farm and were hence not inspected while diseased;
- Disease was not initially reported by owners.

Factors that contributed to the spread of the disease, were:

- Contact between cattle within own herd;
- Contact between herds on adjacent farms;
- Contact between herds at communal diptanks;
- Contact between herds of adjacent diptanks;
- Movement of infected cattle;
- Possible role of game.

The area directly south of the KNP consists of a commercial farming area, where sugarcane, citrus and subtropical fruit are produced intensively, with scattered game and cattle farms in-between, bordering Mozambique. Further south lies a communal area, where sugarcane is also grown, but this area is mainly used for communal cattle farming, bordering Mozambique and Swaziland.

Figure 6. Map showing distribution of cattle in the Nkomazi area at the time of the 2000 FMD outbreaks



METHODS

Surveillance

Following detection of the outbreak in Swaziland, the source of the infection was traced back to Middelburg, Nkomazi and the KNP. Methods used in subsequent surveillance during the ensuing control campaign in Nkomazi are shown in the table.

Method	*Scope*	*Purpose*	*Extent*	*Results*
Physical inspection	All cattle, goats, sheep and pigs	Screening to detect suspect animals	Cattle: 714 804 Goats: 18 402 Sheep: 3 488 Pigs: 16 034 Impala: 54	Suspect animals were detected
Mouth and feet examination	1. A sample from each herd 2. All suspect animals	1. Detection of suspect lesions 2. Estimation of prevalence	Cattle: 9 823 Goats: 991 Sheep: 54 Pigs: 26	1. Suspect lesions detected 2. Prevalence was estimated
Serological specimens	1. A sample from each herd 2. All suspect animals	1. Determination of spread of infection 2. Evaluation of vaccine response 3. Estimation of prevalence	Cattle: 9 262 Goats: 2 794 Sheep: 83 Pigs: 122 Impala: 54	1. Spread of infection was determined 2. Vaccine response was evaluated 3. Prevalence was estimated
Tissue specimens	All animals with suspect lesions	Virus isolation and typing	Cattle: 51	2 viruses were isolated from 1 specimen and typed

The following laboratory tests were done at the Exotic Diseases Division of the Onderstepoort Veterinary Institute:

- Blocking ELISA was done on serological specimens, and since 15 December 2000 the 3-ABC ELISA was done on most positive blocking ELISA specimens.
- Typing ELISA, PCR, virus isolation and virus typing were done on tissue specimens, as applicable.

Serological surveillance

Sample size was determined using the Survey Toolbox software with some degree of simplification. Initially 27 specimens from each farm or diptank area were tested and later on 60 specimens.

The specimens were collected by systematic random sampling. Additionally suspect animals were sampled and recorded as such.

The following variable factors were considered during interpretation of the serological results:

- Previous vaccination coverage;
- Presence of infection;
- Infection prevalence;
- Replication rate of the disease (R_0 » 1,15)
- Virus isolation and typing

The following confounding factors complicated the interpretation of serological results:

- Previous vaccinations
- Test accuracy, especially of 3-ABC ELISA
- Inconsistent results

Serological results were analysed by calculating and assessing the following:

■ Blocking ELISA:

- Proportion of specimens with titres of 1,6 and higher
- Proportion of specimens with titres of 2,0 and higher
- Proportion of specimens with titres of 2,4 and higher
- Mean titre

■ 3-ABC ELISA:

- Proportion of specimens with positive results
- Mean blocking ELISA titre of 3-ABC ELISA positive specimens (insignificant)
- Mean blocking ELISA titre of 3-ABC ELISA negative specimens (insignificant)

Serological results were also analysed in subgroups to assess factors influencing results according to the following:

■ Vaccinations:

- Before any emergency vaccinations
- After the first round of emergency vaccinations
- After the second round of emergency vaccinations

■ Chronology:

- December 2000
- January 2001
- February 2001

Serological results were integrated with other available data:

■ Clinical data

- Contemporary findings (fresh lesions)
- Retrospective findings (old lesions)
- Clinical prevalence

■ Animal movement/contact data

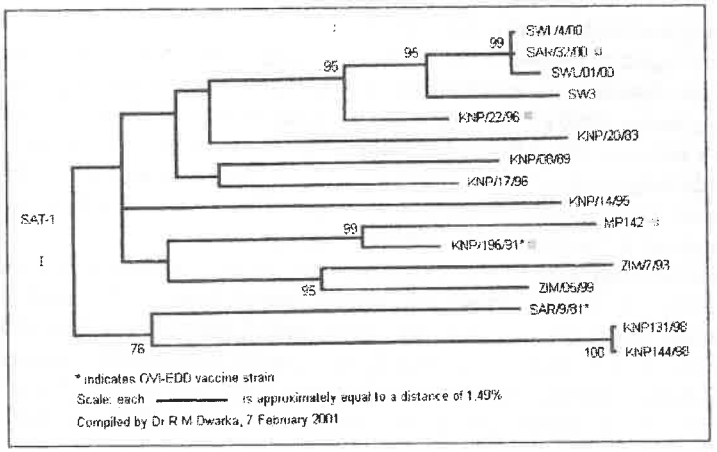
- Stray buffalo movements (stray buffalo register)
- Buffalo/cattle contacts (stray buffalo register)
- Cattle movements (permit registers, other reports)

■ Virological data

- Viruses isolated from Lower Sabie (KNP/22/96), Middelburg (SAR/32/00), Manzini (SWL/4/00) and Zinyane (SW3) (genetically closely related)

- Viruses isolated from Mbiyamiti (KNP/196/91) and Thambokhulu (MP142) (genetically closely related)

Figure 7. Neighbour joining tree depicting VP1 gene relationships in SAT-1 viruses in southern Africa



RESULTS

Spread of the infection

By analysing all available data, a model was developed on the further spread of the infection:

√ The infection spread from Rooigras by movement and contact to the nearby farms Driehoek and Grootboom, both belonging to the same owner and by contact to the adjacent farms Turfbult, Schoonspruit, Umkomaas and Herculina and nearby Symington.

√ Upon detection of the disease amongst his cattle, the owner of Rooigras moved the affected cattle illegally to his other farm Hourvare, from where the disease probably spread by contact to the nearby farms Dadelspruit and Outeniqua.

√ Cattle were moved from Outeniqua to Merribeek, but according to virological, serological and clinical findings, this movement did not spread the infection.

√ Cattle were moved in September 2000 from Umkomaas to Nico's Kamp, belonging to the same owner, hereby spreading the infection.

√ Of the four stray buffalo encountered on Merribeek in August 2000, two were destroyed and two disappearred. The latter probably infected cattle on the adjacent farm Castilhopolis.

√ From September to November 2000 a total of 85 cattle were sold from Castilhopolis to eight diptank areas, of which five (Goba, Masibekela, Sikhwahlane 3, Magudu and Mananga) hereby became infected. Probably by contact the

infection also spread from Castilhopolis to the adjacents farms Quagga and Avondstond, and from there to the adjacent diptank area Brink.

√ In November 2000 a total of 51 cattle were sold from Dadelspruit to four diptank areas, of which three (Thambokhulu, Boschfontein and Jeppe's Rust) hereby became infected. Probably by contact the infection also spread from Dadelspruit to the nearby One Tree Hill.

√ From August to October 2000 a total of 36 cattle were sold from Grootboom to three diptank areas, of which one (Schoemansdal) hereby became infected.

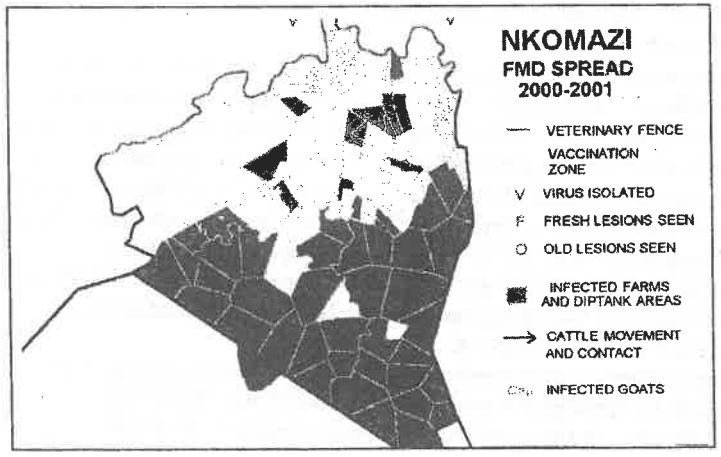
√ In September 2000 a total of 40 cattle were moved from Turfbult to a quarantine camp near White River, from where cattle were moved to a feedlot near Middelburg, some of which illegally and via an abattoir in Barberton. In November 2000 a group of 70 cattle were exported from the feedlot for slaughter at Manzini in Swaziland, where the disease was diagnosed.

√ From the hitherto ten infected diptank areas, the infection eventually spread by contact to 24 adjacent diptank areas at Jeppe's Reef, Buffelspruit, Driekoppies, Middelplaas, Langeloop, Schulzendal, Mgobode 1, Mgobode 2, Mzinti, Phiva, Sihlangu 6, Sihlangu 5, Vukuzenzele, Khombaso, Mabundzeni, Ndindindi, Mbuzini, Mbangwane, Steenbok, Figtree/Mandulo, Mangweni, Albertsnek, Mambane and Walda, and to the adjacent farms Lomati and Amanxala.

√ Vaccination and the isolated locality of four diptank areas (Vlakbult, Sikhwahlane 4, Madakwane and Figtree Breeding Scheme) and the remainder of cattle farms in the commercial farming area, prevent cattle in these areas from becoming infected.

√ Goats became infected in 4 diptank areas (Jeppe's Reef, Khombaso, Mangweni and Walda).

Figure 8. Map of Nkomazi showing eventual spread of FMD and clinical and virological findings



In summary 34 out of 38 diptank areas (89 per cent) in the communal area and 18 out of 56 cattle farms (32 per cent) in the commercial farming area eventually became infected. A series of maps illustrating the spread of the infection were produced and are available.

Vaccine response

In the Nkomazi area two emergency rounds of cattle vaccination were done, the interval ranging from 14 to 27 days and the mean interval being 20 days.

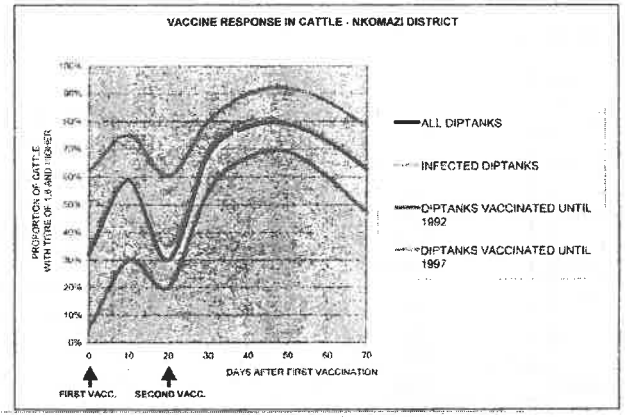
The following aspects were evaluated (only in the communal area):

- Immune response following first vaccination
- Immune response following second vaccination
- The influence of previous vaccinations (until 1992 and 1997 respectively)
- The influence of infection

Interpretation of results for the purpose of evaluating vaccine response was confounded by the presence of infection. Only four out of 38 diptank areas remained free of infection, limiting the available data on vaccine response in absence of infection. However, the following results were obtained:

- After the first vaccination, response was generally weak, but much stronger in those diptank areas where vaccination was practiced until 1997 than in those where vaccination was terminated in 1992.
- After the second vaccination, response was much better, and even more so in those diptank areas where vaccination was practiced until 1997.
- Throughout the period response in diptank areas with infected cattle was slightly better than on the whole.

Figure 9. Vaccine response in cattle in the Nkomazi district



Estimation of prevalence

Serological (3-ABC ELISA) and clinical data (mouth examinations) was analysed in an attempt to estimate the period prevalence of FMD in the Nkomazi

area. In this way it was initially estimated that 12 335 out of 55 610 communal cattle (22 per cent) and 749 out of 7 542 commercial cattle (10 per cent) contracted FMD infection during the outbreaks. These calculations did however not take the accuracy of the tests into consideration. Since both tests have low sensitivity, these figures were regarded as minimum figures.

The true prevalence and predictive values of the 3-ABC ELISA can be calculated by analysing the results of all the tests that were done per sector as follows:

Given: 3-ABC ELISA : sensitivity = 50%, specificity = 99%
Figures in italics were calculated

COMMUNAL CATTLE		INFECTION +	-	total
3-ABC ELISA	+	*385*	*12*	397
	-	*385*	*1 209*	1 594
	total	*770*	*1 221*	1 991

estimated prevalence = 20%
true prevalence = *39%*
Predictive value...
of positive test = *97%*
of negative test = *76%*

COMMERCIAL CATTLE		INFECTION +	-	total
3-ABC ELISA	+	*41*	*9*	50
	-	*41*	*915*	956
	total	*82*	*924*	1 006

estimated prevalence = 5%
true prevalence = *8%*
Predictive value...
of positive test = *82%*
of negative test = *96%*

When these calculations were done per farm or diptank in stead of sector, a true prevalence of 42 per cent was obtained for the communal area and 19 per cent for the commercial area. After calculation of true prevalences per sector, the estimated numbers of infected cattle were adjusted to 23 130 communal and 1 435 commercial cattle. Henceforth the sensitivity and predictive values of the retrospective mouth examinations were calculated:

Assumption: specificity of retrospective mouth examinations is 100%

COMMUNAL CATTLE		INFECTION +	-	total
MOUTH EXAMINATION	+	77	0	77
	-	*905*	*1 558*	2 463
	total	*982*	*1 558*	2 540

true prevalence = 39%
sensitivity = 8%
Predictive value...
of positive test = 100%
of negative test = *63%*

COMMERCIAL CATTLE		INFECTION +	-	total
MOUTH EXAMINATION	+	15	0	15
	-	108	1 391	1 499
	total	123	1 391	1 514

true prevalence = 8%
sensitivity = 44%
Predictive value...
of positive test = 100%
of negative test = *93%*

CONCLUSION

- Integrated interpretation of serological, clinical, vaccination and animal movement data is very useful in forward and backward tracing during a FMD epidemic.
- Time constraints and priorities during the FMD control campaign disallow proper analysis of all available data, and necessitates a conservative approach e.g. by declaring inclusive quarantine and surveillance zones.
- In communal areas spread of FMD is much faster and prevalence much higher than in commercial areas.
- Negative results of both the 3-ABC ELISA and mouth examinations have low predictive values when prevalence is high such as was the case in the Nkomazi communal area.
- Retrospective analysis of data may indicate risks that should be considered when control measures are reviewed.
- Serological vaccine response evaluation is complicated in the presence of infection.
- Vaccination coverage does not influence serological results significantly.
- Serological and clinical prevalence may differ vastly due to lower virulence or higher immunity.
- Lack of surveillance in game may lead to underestimation of the role of game in spread of FMD.

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AN EXAMINATION OF THE INITIAL SPATIAL DISTRIBUTION PATTERNS OF FOOT AND MOUTH DISEASE AT DIP TANKS DURING THE 2001 BUSHBUCKRIDGE OUTBREAK IN SOUTH AFRICA

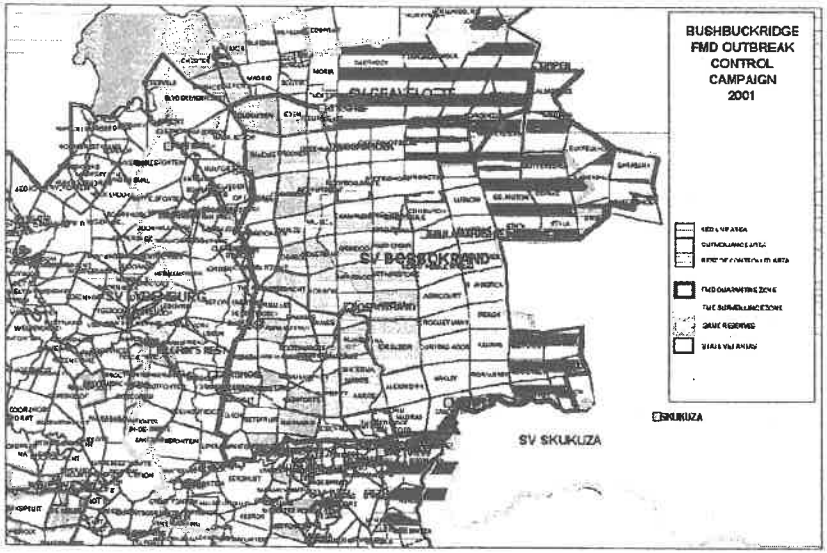
E DYASON

SUMMARY

During the first two weeks after the confirmation of Foot and Mouth disease (FMD) in the Bushbuckridge area random blood samples were taken from cattle at each diptank in the area. FMD vaccinations are done regularly in the vaccination zone, while cattle in the other control zones were vaccinated to a certain extent in the past. The movement of vaccinated cattle from vaccination zones to other zones does happen. This brings about the presence of previously vaccinated cattle widely distributed throughout the area. Because of this practise the results received could not be interpreted individually. The percentage cattle with FMD titers above certain values were then determined per dip tank and plotted onto a map of the area. This exercise clearly showed where the disease started.

INTRODUCTION

Foot and Mouth disease (SAT 2) was confirmed on 1 February 2001 on the



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farm Orinoco 233 KU in the Mhala district. Veterinary services started a full-scale inspection of the entire Bushbuckridge area. The 22 inspection teams took random blood samples at each diptank or farm within the quarantine area. These samples had to indicate the presence, origin and spread of the infection.

The cattle in the vaccination zone are vaccinated twice per year against FMD and were vaccinated during October 2000. It was expected that serological reactions would be present in these cattle. Herds in the diptank areas adjacent to the vaccination zone were vaccinated for the last time in 1998. Some low-level reactions were expected at these dip tanks. The movement of vaccinated cattle within the communal area of Bushbuckridge is allowed under strict veterinary control. I expected to pick up individual reactions in the unvaccinated zones because of this practice.

METHODS

Sample taking and results

A total of 2 851 blood samples were taken at 131 diptanks or herds throughout the area. These samples were sent to the exotic diseases section of Onderstepoort Veterinary Institute. Using the Blocking Elisa test the samples were tested for serological reactions against three FMD strains, namely: SAT 1, SAT 2 and SAT 3. Only reactions against SAT 2 were considered because the outbreak was confirmed as being SAT 2. Of the 131 diptanks, 26 diptanks or herds were in the vaccination zone and all but one herd showed serological reactions. In the non-vaccinated zone only 32 diptanks or herds showed completely negative results. The remaining 72 diptanks or herds were widely distributed throughout the area and the cattle showed reactions with various values. No pattern or picture could be determined from these individual results nor could the results differentiate between previously vaccinated cattle and the current disease outbreak.

Alternative calculations and plotting

To establish a pattern or picture from all these serological reactions I decided to work with percentages of positive reactions per herd, rather than individual reactions.

All the herds were tabled and the number of positive reactions (SAT 2) equal to or above a value of 1.6, which is considered a positive reaction, were calculated and worked out as a percentage of the total number of samples taken. The same calculations were done with a value of 2.0.

An example of calculations:

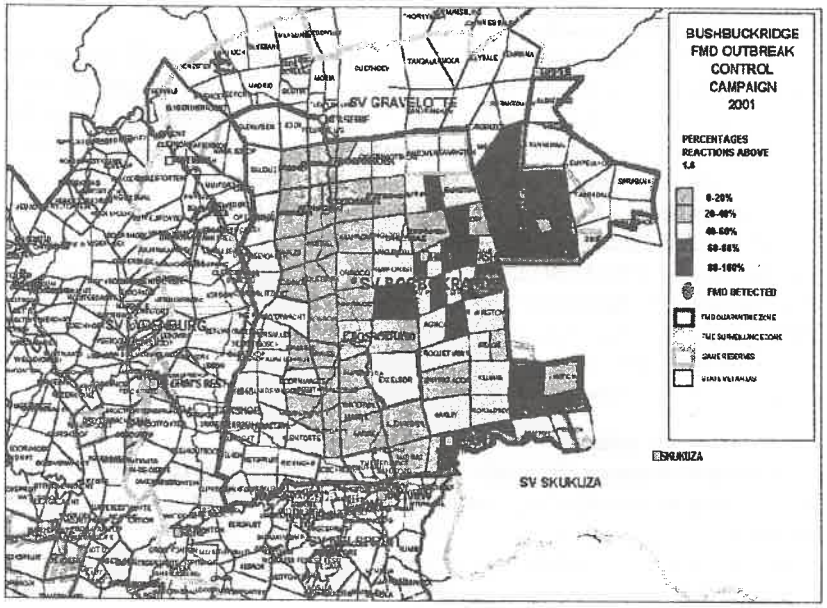
Farm	*Samples*	*> 1.6*	*%*	*> 2.0*	*%*
Acornhoek	20	10	50	1	5
Agincourt	25	20	80	14	56
Athol	20	20	100	13	65
MP Stream	27	26	96	24	89

Colour codes were given to the different percentages namely:

0-20%	green
20-40%	orange
40-60%	yellow
60-80%	red
80-100%	blue

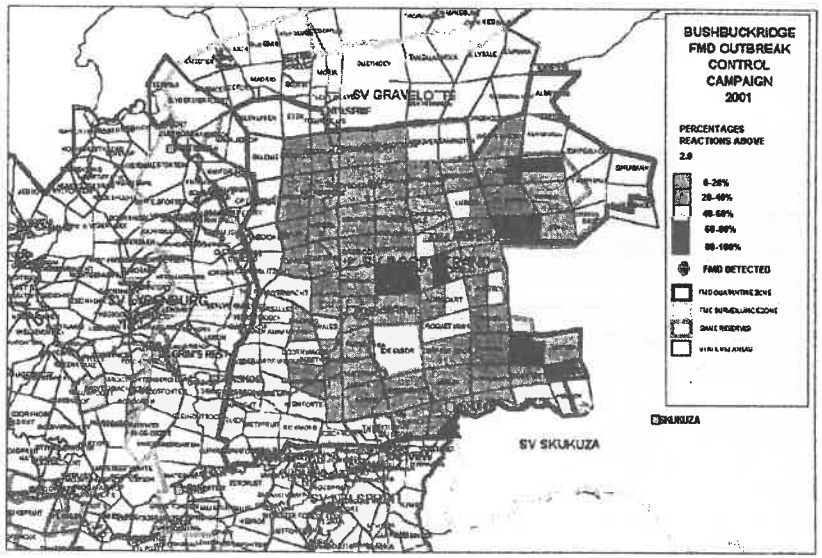
RESULTS

To picture the difference in the percentage of positive SAT 2 reactions / diptank the colours where plotted onto the map of the area, according to the location of the diptanks. The map with the reactions ≥ 1.6 did not indicate much except that there is good immunity in the vaccination zone.



The map with the reactions ≥ 2.0 gave a clear picture. In the surveillance zone two diptanks showed above 80 per cent positive reactions and one diptank showed above 60 per cent positive reactions. All three diptanks are in the center of the area. It is important to note that in the vaccination zone no diptank had above 80 per cent reactions.

These percentages of positive reactions per diptank indicated where the FMD was and where the cattle had the chance to sero-convert.



DISCUSSION

The individual reactions were very confusing. The probable cause was that many more than expected vaccinated cattle were moved from the vaccination zone to the unvaccinated zone.

The concept of working with percentages did indicate a workable solution. One of these centre farms was the locality where three-week-old foot lesions were detected one day after the confirmation of the disease in the area. With the investigation into a possible source of the infection it was reported by environmental officials that two buffalo which had escaped from a private reserve had been in this centre area about six weeks before the outbreak was confirmed.

CONCLUSION

Where individual reactions are very confusing, specifically in situations where animals were vaccinated against the disease under investigation, the calculation of percentages of infected animals per dip tank or herd is a possible way of interpreting the serological reactions.

POSTERS

A FIELD EVALUATION OF THREE TRYPANOSOMOSIS CONTROL STRATEGIES IN KWAZULU-NATAL, SOUTH AFRICA

F R EMSLIE[1], B GUMMOW and J R NEL

Rural subsistence farming practices are the primary agricultural activity in northeastern KwaZulu-Natal, South Africa. Since Bruce first identified *T.brucei brucei* in the late 1800's, cattle in this area have been affected by tsetse-borne trypanosome infections. Approximately 120 000 cattle fall within a tsetse (*G.austeni* and *G.brevipalpis*) belt common to Mozambique and South Africa. Between 1991 and 1994 cattle in this area were treated with homidium bromide, and dipped with cyhalothrin, in an attempt to control trypanosomosis. However since 1994 no control measures were implemented and trypanosomosis re-emerged as a threat to animal health.

In order to determine the optimum control measure available, a longitudinal incidence study was conducted to evaluate three possible control options.

Four sentinel herds were selected from populations exposed to similar trypanosome challenges. The baseline trypanosome incidence rate was determined for each herd, after which each herd was subjected to a different trypanosome control measure. Two of the herds were subjected to topical pyrethroid treatment (Cyfluthrin pour-on and Flumethrin plunge-dip) as a vector control measures, one herd was treated six weekly with an injectable trypanocidal drug (isometamidium hydrochloride), and one herd served as an untreated control group. Monthly incidence rates were determined using the 'dark-ground buffy smear technique'.

The monthly incidence rates were standardized in order to account for variation in trypanosomosis challenge between the four herds. The standardized rates were then compared and the impact of the control strategies was quantified using the Area Under The Curve method. The cost efficacy of each control strategy was evaluated based on a partial budget system.

Both the cyfluthrin pour-on, and the injectable trypanocide, were cost effective and had a dramatic trypanosomosis control effect with the pour-on having the greater impact/control. The flumethrin plunge-dip displayed moderate trypanosomosis control properties, but proved not to be cost effective!

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SURVEY OF FACTORS INFLUENCING KID SURVIVAL IN SMALL SCALE GOAT FLOCKS ON COMMUNAL LANDS IN JERICHO

C M E MCCRINDLE[1], P J SEBEI[1] and E C WEBB[2]

The aim of the study was to assess factors affecting the survival to weaning of kids born to does kept on communal grazing lands in Jericho. This is a low-input, low-output goat keeping system. Extension messages would be developed, based on structured interviews, field observations over a 12-month period, statistical analysis of data, as well as scenario planning. Initially 20 farmers were interviewed. Of these 14 farmers with 223 goats remained in the trail for the full period. The average number of adult goats per flock was 13.3.

Both quantitative and qualitative analysis of data was performed. Pearson correlation and regression was used to examine the significance of variables that may have influenced kid survival. The only significant determinant was the incidence of internal parasites, measured as eggs per gram (*Haemonchus and Trichuris* spp.) and oocysts per gram (*Eimeria* spp.) in monthly pooled samples of faeces. Correlation was significant at the 0.01 level (two tailed).

There was no correlation with precipitation and daily temperatures; nor with nutrition, as measured by body condition scores and veld evaluation over the full trial period. The one farmer who milked his does, had the highest mortality rate measured as a percentage of kids born (range 18-75 per cent). Body condition score could not be related to management practices except in the case of one farmer who only allowed goats out of the kraal for 3 hours a day (range 3-8 hours). The phenomenon of peri-parturient shedding of nematode eggs was also observed. Qualitative evaluation of housing showed that 92 per cent were sub-optimal (<2.5 on a scale of 1 to 5) and that only two farmers removed faeces from the kraals.

High levels of internal parasites may be caused by poor hygiene in the kraals as well as a lack of parasite control. The suggested extension message was therefore to shift the kraal to another location just before the kidding seasons. The old kraal could then be used for planting or the rotted manure packaged and sold as fertiliser. Does should be de-wormed strategically, once a year (possibly at the beginning of the dry season – May) and kids should be de-wormed once before five months of age (the average age for natural weaning). Scenario planning and cost-benefit analysis using Microsoft Excel indicated that this strategy, rather than improved nutrition, would be most likely to optimise the system and increase margins.

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WILDLIFE TUBERCULOSIS, BOVINE TUBERCULOSIS AND ZOONOTIC TUBERCULOSIS – POTENTIAL THREATS TO HUMAN HEALTH IN RURAL AFRICAN COMMUNITIES?

A MICHEL[1]

Following the implementation of a National Tuberculosis Control and Eradication Scheme in 1969, the prevalence of tuberculosis in South African commercial cattle herds has dropped sharply to below 0.01 per cent. This does, however, not include cattle herds belonging to rural communities and subsistence farmers. It further excludes the tuberculosis epidemics currently experienced in South Africa's two major game reserves, the Kruger National Park (KNP) and Hluhluwe-Umfolozi Park (HUP). It is estimated that bovine tuberculosis spilled over from cattle to African buffalo (*Syncerus caffer*) in the KNP via the wildlife-livestock interface approximately 40 years ago. It has since spread among buffalo herds in the southern and central regions of KNP and was more recently also diagnosed in the northern areas. With increasing prevalence rates in buffalo, which are regarded as maintenance hosts for tuberculosis, spillover to other animal species such as lion (*Panthera leo*), leopard (*Panthera pardus*), Cheetah (*Acinonyx jubatus*) hyena (*Crocuta crocuta*), chacma baboon (*Papio ursinus*), greater kudu (*Tragelaphus strepsiceros*), warthog (*Phacochoerus aethiopicus*)and honey badger (*Mellivora capensis*) became evident. If uncontrolled, this epidemic is expected to spread over the entire areas of both the KNP and HUP, affecting many more species over time. Both parks are mostly bordered by communal farmland of resource poor farming communities and only few commercial farms. Despite the erection of adequate game fencing over the full length of both parks, this barrier can only partially prevent contact between wild animals and domestic livestock. Under these conditions the risk of tuberculosis spillover into domestic livestock outside the parks is increasing and cannot be ignored.

As mentioned, communal cattle herds are not regularly tested for bovine tuberculosis. Therefore, once present in a herd, the infection is allowed to progress into the advanced stages of disease, with animals developing open lesions and shedding bacilli mainly in milk and aerosols. These are at the same time the main routes of transmitting zoonotic tuberculosis, in particular because milk is primarily consumed unpasteurised. The high incidence of HIV/AIDS in South Africa is a another complicating factor potentially contributing to the transmission of zoonotic tuberculosis. Immunocompromised patients, especially children, are more likely to contract M. bovis infection than healthy individuals. Presently the role of zoonotic tuberculosis in humans in South Africa is unknown, but both the raging HIV/AIDS and wildlife tuberculosis epidemics urge for a joint veterinary/medical approach to assess the current situation.

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SCREENING TEST FOR A CONGENITAL MYASTHENIC SYNDROME IN CATTLE

P N THOMPSON, O K STEINLEIN, E VAN DYK, S KRANER, C HARPER, A J GUTHRIE, A NEL and E BELL

A congenital myasthenic syndrome was first reported in cattle in 1998, when progressive muscular weakness was observed in four half-sibling Red Brahman calves (Thompson, 1998). The genetic basis for the disease is a homozygous 20 base pair (bp) deletion (470del20) in exon 5 of the gene (bov*CHRNE*) coding for the epsilon subunit of the nicotinic acetylcholine receptor (AChR) (Kraner et al, 2002). This mutation is predicted to result in a non-functional allele and the inability to produce functional adult-type AChR. The mutation is inherited in an autosomal recessive manner.

Blood samples were collected in EDTA from 30 Red Brahmans from various farms. These included several known carrier animals, close relatives of the carriers and unrelated animals. The blood was stored and the DNA fixed on FTA(R) paper. Exon 5 of the bov*CHRNE* gene was amplified using specially designed forward and reverse labelled primers. Amplification products were then sized on an ABI 310 sequencer. A single 211 bp peak indicated a homozygous normal animal. Two peaks at 211 bp and 191 bp indicated a heterozygous carrier animal. In addition, DNA was extracted from the blood samples for screening at the Institute for Human Genetics, Bonn, where the mutation was originally identified. Screening was done by amplification of exon 5 and separation of fragment sizes by gel electrophoresis. The identities of the two fragments were confirmed by sequencing.

The validation of this screening test provides a rapid, inexpensive and accurate means of identifying carriers of the mutation responsible for this congenital myasthenic syndrome. This will allow future studies of the prevalence of carrier animals and of the clinical and economic importance of the condition. In particular, investigation into the possible involvement of this mutation in cases of idiopathic calf mortality are warranted.

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PROTECTION OF PIGS WITH AN EMERGENCY FOOT AND MOUTH DISEASE SAT-1 OIL VACCINE

B DUNGU[1,2], C PHIRI[1], P KLOECK[3], J ESTERHUYSEN[1], A BASTOS[4], K BOSHOFF[1] and W VOSLOO[1]

An outbreak of foot-and-mouth disease (FMD) due to SAT-1 occurred among cattle at a feedlot in the Middelburg District of Mpumalanga Province, South Africa in November 2000. The infection was confined to a cattle section of the feedlot housing 15 674 cattle situated 275 meters from 51 159 pigs and 2 471 sheep present on the farm during the outbreak. As part of the control and prevention strategy, emergency vaccination was carried out with a trivalent saponin-alhydrogel adjuvenated SAT vaccine for cattle and sheep and a monovalent oil adjuvenated SAT-1 vaccine in pigs. Genetic analysis showed that the FMD virus in this outbreak was the cause of the cases of FMD reported at an abattoir in Swaziland during the same period and to specific buffalo genotypes in the Kruger National Park but different from those in the 1999 outbreaks in the sub-region (Zimbabwe, Zambia and Tanzania). The outbreak strain was also different from the SAT-1 outbreak affecting buffalo on a farm in Phalaborwa, Limpopo Province of South Africa in 2000. Serological studies showed a good antigenic relationship between the outbreak virus and the SAT-1 strains in the vaccine. A group of vaccinated pigs that were later transferred to the Exotic Diseases Division of the ARC-OVI for challenge studies showed that the emergency vaccination conferred protection against the outbreak strain. However, the outbreak never spread to the piggery as was subsequently demonstrated by an absence of antibodies to the non-structural proteins of FMD virus.

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CHARACTERISATION OF THE VIRUS INVOLVED IN THE 2000 AND 2001 FOOT AND MOUTH DISEASE OUTBREAKS IN RWANDA

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Foot and Mouth Disease (FMD) is a highly contagious acute viral infection of cloven-hoofed animals carrying severe economic implications. Rwanda has experienced several outbreaks in the past years, with several possible factors playing a role in the epidemiology of the disease. The aim of the study was to confirm the presence of FMD in Rwanda, isolate and characterize the outbreak strains, determine the extent of the infection in order to guide the design of a more representative surveillance campaign and formulate a custom made vaccine for the country. Serum, blood, epithelium and oesophago-pharyngeal (OP) fluids (probang) were collected from the clinically infected cattle, while serum was collected from an in-contact pig and goat in Kubungo. Sampling was done in the Gisenyi, Kibungo, Umutara and Gashora regions, while the Rubirizi veterinary laboratory supplied serum from Kigali.

Serological samples tested positive for antibodies to SAT (Southern African Territories) type-1, - 2, - 3 and serotype O, indicating that more than one serotype have been involved in disease episodes in the country. SAT-2 viruses were isolated from OP samples during 2000 and 2001 and the close genetic relationships between the isolates indicated that it was the same epizootic, caused by the same virus over a period of at least one year. These viruses were unrelated to any other virus previously investigated at this laboratory, indicating that a unique vaccine should be developed for this region.

It was concluded that the epidemiology of FMD in Rwanda is still poorly understood and that a more representative national surveillance should be conducted in which the role of wildlife is also investigated. The presence of seropositive animals in regions where the disease has not been reported previously indicated that the problem was more extensive than initially thought. The presence of antibodies to SAT-2 in the majority of the animals suggested the predominance of this serotype in the country. A second mission was advocated in which the government was to be shown the possible magnitude of their FMD problem and convinced to undertake a countrywide surveillance and use a custom made vaccine.

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SEROLOGICAL SURVEY TO CONFIRM THE FOOT AND MOUTH DISEASE FREE STATUS OF SOUTH AFRICA AFTER THE 2000/2001 OUTBREAKS OF THE DISEASE

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During 2000/2001 South Africa experienced its first outbreaks of foot and mouth disease outside the foot and mouth disease (FMD) control zone since 1957. The first outbreak was caused by serotype O, which had never previously been recorded in the country. This outbreak in KwaZulu-Natal affected a small part of the Camperdown district. An outbreak of SAT-1 subsequently occurred in a feedlot in the Middelburg district of Mpumalanga. Both outbreaks were efficiently controlled and no spread beyond the outbreak foci occurred.

However, it was necessary to perform a countrywide serological survey to confirm that the disease had not spread to other parts of the country in order to regain OIE FMD-free status without vaccination and to regain trust by trade partners. The survey was planned using a two-stage sampling strategy with stratification according to provinces. The FMD control zone, and land parcels where animals would normally not occur, such as mining areas, water masses, urban areas etc., were not included in the sampling frame. Only cattle were sampled, as it was assumed that they were the most susceptible domestic animal species to FMD infection and because they show clinical disease more readily. In regions where cattle numbers were extremely low sheep were sampled in place of cattle.

Several assumptions were made for each stage of the sampling strategy. Based on these 62 land parcels per province were randomly chosen according to a grid reference system and 20 animals were systematically randomly bled on each farm. Since the communal farming areas were perceived as an unknown risk, more of these areas were included in the final study.

A total of 10 826 animals were sampled on 634 farms and no animals tested positive for antibodies to SAT-1, SAT-2 and serotype O. Results from an independent pig survey, buffalo tested upon movement, as well as results generated for imports and exports were added to the survey to increase the validity of the survey. The findings of the survey resulted in the reinstatement of the country's previous OIE zoned disease free status without vaccination on 31 May 2002.

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