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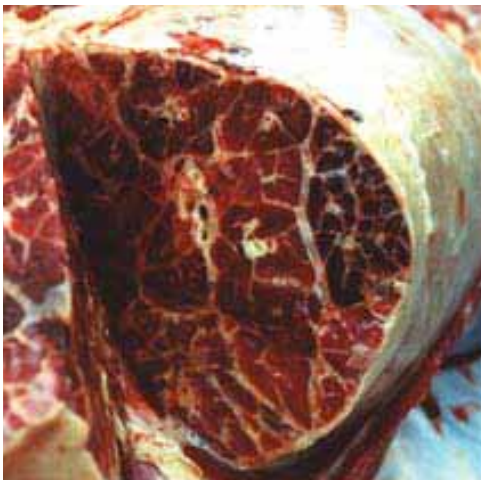
FAO ANIMAL PRODUCTION AND HEALTH


proceedings

CBPP CONTROL: ANTIBIOTICS TO THE RESCUE?

FAO-OIE-AU/IBAR-IAEA
Consultative Group Meeting on CBPP in Africa
Rome, 6-8 November 2006

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2007

Contents

Welcome address

Dr Joseph Domenech[1]

Dear Colleagues,

On behalf of FAO, I welcome you all to Rome and to the 4th meeting of the Consultative Group on contagious bovine pleuropneumonia (CBPP). A special welcome is extended to Prof. Vincenzo Caporale, who is representing the World Organisation for Animal Health (OIE), and to Dr El Sawalhy who is representing the African Union-Inter African Bureau for Animal Resources (AU-IBAR). I thank Dr William Amanfu – Animal Health Officer of FAO for organizing this meeting.

It has been three years since the last meeting of this Consultative Group and, since then, many things have changed in the field and in research on CBPP. The purpose of this meeting is to review and reflect on: the situation of the disease in Africa; new developments in research; and the use and efficacy of antibiotics for the treatment of CBPP.

Within the context of other transboundary animal diseases in Africa such as highly pathogenic avian influenza (HPAI) and *peste des petits ruminants* (PPR), CBPP remains one of the major diseases of livestock in Africa today where it causes significant economical losses and is a barrier to trade. Control programmes are difficult to implement in many areas, specifically due to mobility in pastoral animal husbandry, lack of animal identification systems, lack of human and financial resources especially for vaccination or stamping-out campaigns or, in some places, civil unrest. Therefore, CBPP remains widespread in eastern, central and western Africa. Nevertheless, CBPP control is possible in Africa as exemplified by eradication of the disease from Botswana during the 1990s and control in Guinea and Senegal.

The tools necessary for surveillance and control are available but other areas that require improvement remain, such as diagnostic tools, surveillance systems, and strengthening of veterinary services. The Pan African Rinderpest Campaign (PARC) and Pan African Control of Epizootics (PACE) programmes have supported diagnostic and surveillance capacities for transboundary animal diseases including CBPP, at the national and regional levels. A lot still remains to be done. PACE comes to an end in February 2007. The global HPAI crisis has shown the need to reinforce prevention and control of transboundary animal diseases and FAO's Emergency Prevention System for the progressive control of transboundary animal disease (EMPRES) programme continues to direct its efforts at the international, regional and national levels to support early detection, reporting, early warning and rapid response. The global framework for transboundary animal diseases (GF-TADS) initiative between FAO and OIE has been undertaken to develop better synergies between our organizations towards these goals.

Antibiotic use will be one of the major issues to be addressed during this expert consultation. It is well known that in Africa antibiotic use is widespread in the treatment of animal diseases but this is done without appropriate controls and methods.

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[1] CVO/Chief, FAO Animal Health Service

Vaccination is one of the most efficient tools for disease control if implemented appropriately. There were some questions five years ago with regard to the efficacy and inocuity of T1/44 but these have now been solved. Even so, the current vaccines are not ideal and there is a great need to improve vaccines for CBPP. There have been some scientific advances afforded by the use of new technologies that have allowed a better understanding of the immunology of the disease and the response to vaccination. Advances in sequencing technologies have made it possible to rapidly assess strains at the genetic level.

Research groups will inform us of the state of the art and the prospects. The vast research programme which has recently been prepared under the umbrella of the African Livestock (ALive) initiative will be presented and discussed during the meeting. Development of new vaccines and diagnostic tools, economic assessment and research and delivery systems are included.

This consultation has a very busy agenda. I am sure you will work very hard and will come to clear and concrete recommendations to guide the international and African communities in the fight to control and prevent CBPP.

I again thank all experts present here as well as the OIE, IBAR and FAO/IAEA Joint Division for their support. I wish you fruitful discussions and a very pleasant stay in FAO and in Rome.

Opening Address

Samuel Jutzi[1]

Distinguished Scientists, Researchers on contagious bovine pleuropneumonia (CBPP) disease, Chief Veterinary Officers and Field Officers, ladies and gentlemen.

It gives me great pleasure to open the 4th Consultative Group CBPP meeting at the Food and Agriculture Organization (FAO) Headquarters in Rome. The Consultative Group CBPP meeting which involves the World Organization for Animal Health (OIE), the African Union–Inter African Bureau for Animal Resources (AU-IBAR), the FAO/IAEA Joint Division and the FAO, has afforded these organizations and the international scientific community an excellent technical platform for exchange of ideas and synthesis of strategic initiatives aimed at the progressive control of CBPP. Currently, there appears to be no such platform for a coordinated exchange of views on this disease anywhere and FAO will, in as much as its resources allow, continue to support such dialogue in the belief that this disease, which is still a major constraint to cattle production in Africa, will be controlled and where feasible, eradicated so as to enhance food security and improve people's livelihoods on the continent and elsewhere.

FAO takes its commitment to animal-disease control seriously and together with its partners, will support initiatives directed at achieving this objective. The recent launch of the Crisis Management Centre (CMC) at FAO Rome, in collaboration with the OIE, United States Department of Agriculture (USDA) and other donors, is yet another demonstration of FAO and its international partners' commitment to managing animal-disease outbreaks, especially those of transboundary nature. The centre will provide technical and operational expertise in responding rapidly to outbreaks of transboundary animal diseases and in disease trend analysis. Although the current focus of the CMC is on highly pathogenic avian influenza (HPAI), extension of activities of the centre to cover other transboundary animal diseases such as CBPP, foot and mouth disease and others, is clearly foreseen. Likewise, the Africa livestock (ALive) platform, which is a multipartner programme initiated by the World Bank in conjunction with its international partners such as FAO, OIE, AU-IBAR and others for livestock-related initiatives in Africa geared towards improved livestock productivity and poverty reduction, is currently working on proposals aimed at addressing some of the research priorities in CBPP disease.

Ladies and gentlemen, this meeting helps to refocus attention on this equally important transboundary animal disease – CBPP – that also requires our concerted attention and efforts, despite our involvement in handling the global situation of HPAI.

Contagious bovine pleuropneumonia has remained a major scourge on the African continent despite the fact that it has been eradicated from most parts of the world. The situation in Africa is all the more important and requires coordinated efforts at resolution

[1] Director, FAO Animal Production and Health Division

in view of the fact that most veterinary services have deteriorated over the years leading to a weakening in the chain of command critical for animal-disease reporting and effective response. Public good services must be resuscitated with regards to the control of CBPP and other transboundary animal diseases. The control of some transboundary animal diseases in the past was most successful in much of Africa, because veterinary services were much stronger then than they are today. They were capable of mounting successful vaccination campaigns and carried out animal-disease investigative work, some level of research and operated within a legislative framework for animal-disease control in respective countries. It must however be pointed out (as you all know) that CBPP control/eradication is technically far more difficult. The control of CBPP requires the effective functioning of all elements of a well-functioning official veterinary service. Indeed, the surveillance and control of CBPP can be taken as a marker of the strength of the veterinary service in an affected country. For CBPP disease to be effectively managed on the continent, the structures of veterinary services must therefore be improved.

I notice that the overall theme of this year's CBPP meeting is "Control of CBPP: antibiotics to the rescue?" Whilst this theme may appear to be provocative, it seems to me that it is time for a vigorous scientific debate and structured scientific approach to answering some of the questions frequently asked on the therapeutic effectiveness of antibiotics in managing CBPP disease. Antibiotic use is a fact of life, especially in pastoral settings, contrary to official policy and the requirements of the OIE Terrestrial Animal Health Code. Our research efforts must attempt to answer some of the missing gaps in scientific knowledge in the use or non-use of antibiotics for CBPP disease management, particularly in pastoral settings. Antibiotic residues and implications for the emergence of antibiotic resistant micro-organisms, not only for the causative agent of CBPP – *Mycoplasma mycoides* subsp. *mycoides* SC variant – but also other animal and human pathogens, are factors that require serious consideration.

It is reassuring to note that other research efforts such as vaccine developments and improved diagnostic tests will be addressed during your deliberations. Recent advances in science have opened up tremendous opportunities and avenues for developing new CBPP vaccines that could provide longer-lasting immunity and are thermo tolerant. We must critically examine all options in the control of CBPP with a view to assisting countries or zones of countries in the management of the disease through the elaboration of holistic control options based on specific epidemiological situation of the disease.

I wish you very fruitful deliberations and hope that the outputs from this meeting will advance the control/eradication of CBPP in Africa. It is my pleasure to declare the 4th Joint FAO/OIE/AU-IBAR/IAEA Consultative Group Meeting on CBPP formally open.

Thank you

Principal objectives of the consultation and expected outputs

Juan Lubroth[1]

Over the years, FAO in collaboration with its partner institutions AU-IBAR, joint division of FAO/IAEA and the OIE have tried as much as possible to maintain the general format of the CBPP consultative process, since this offers a unique platform for synthesizing scientific and field experience into workable alternatives for dealing with CBPP outbreaks. As has been pointed out by the previous speakers, the use of antibiotics to treat CBPP disease is known to all of us in this forum. We are aware that this activity in the field is against the provisions of the OIE Terrestrial Animal Health Code. There are those who advocate for the use of antibiotics to control CBPP because it principally reduces mortality caused by the disease and there are those who are against the use of antibiotics in CBPP because it produces chronic asymptomatic carriers responsible for the spread of the disease. Those of us in the scientific community owe it a duty to provide a science-based decision platform to assist the rural pastoralist whose livelihood and sheer survival is dependent mainly on cattle production.

The principal objective of this consultation therefore is to review and discuss the current scientific information available in the control of CBPP as it relates to the use of antibiotics in controlling the disease, recognizing the initiatives of AU-IBAR/PACE, and the recommendations of the 3rd CBPP Consultative Group Meeting held in Rome in 2003.

The expected outputs are:

- an analysis of available technical information on the *in vitro* and *in vivo* use of various classes of antibiotics in the management of CBPP and an indication of critical gaps in knowledge that need to be addressed;
- parameters/criteria for the use of any class of antibiotics if deemed appropriate for use in controlling CBPP;
- private sector/institutional partnerships in research and development efforts for long-term strategic studies to resolve the issue of antibiotics use in controlling CBPP disease. This is to generate interest in research and advocacy for funds for further studies on the pathogenesis of CBPP and the role of antibiotics in the control of the disease;
- a review of current information on other CBPP-control strategies
- a statement of purpose with regards to the use or non-use of antibiotics in CBPP management.

In conclusion, this consultation is made to stimulate discussions on the subject of use/or non-use of antibiotics and to indicate areas where research could be intensified to address missing gaps in knowledge. It does not necessarily suggest a shift in official FAO policy on the use of antibiotics in treating CBPP disease.

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[1] Senior Officer, FAO, EMPRES

The use of antibiotics for CBPP control: the challenges

William Amanfu[1]

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an insidious pneumonic disease of cattle sometimes referred to as lung sickness. The causative agent of CBPP is *Mycoplasma mycoides* subspecies *mycoides* SC variant (*Mmm*SC). Phylogenetically, the organism is a member of the *Mycoplasma mycoides* cluster which are pathogens of ruminants. CBPP is primarily a disease of cattle. Both *Bos taurus* and *Bos indicus (zebu)* breeds are fully susceptible. CBPP is characterized by the presence of sero-fibrinous interlobular oedema and hepatization giving a marbled appearance to the lung in acute to subacute cases and encapsulated lesions (sequestra) in the lungs of some chronically infected cattle. Joint infections are common in calves. The disease is present in Africa and until recently, in the Iberian Peninsula of southern Europe. It has been eradicated from North America, Europe, Australia and most parts of Asia.

In Africa, CBPP is found in an area south of the Sahara from the Tropic of Cancer to the Tropic of Capricorn and from the Atlantic to the Indian Ocean. In the early 1970s, the CBPP disease situation appeared to be under control. As such, the interest of veterinary authorities, regional and international animal health organizations shifted to priorities other than the control of CBPP. However, after almost 20 years of respite, CBPP made a spectacular come-back on two major fronts – one in the east of the continent and the other in the south. Almost at the same time, there was a resurgence of the disease in previously known endemic areas of West Africa. Endemic infection extends throughout the pastoral herds of much of western, central and eastern Africa and in Angola, northern Namibia and Zambia. Botswana, Comoros, Lesotho, Madagascar, Malawi, Mozambique, South Africa, Swaziland and Zimbabwe are currently (2006) free from the disease. CBPP represents a major constraint to cattle production in Africa and is regarded as the most serious infectious animal disease affecting cattle on the continent.

The key elements of CBPP control like many other animal disease epizootics are: early detection; rapid response to reduce the level of infection; and eradication of the disease in the shortest possible time. With CBPP, this is achieved by a "stamping-out" policy that requires the slaughter of infected and in-contact herds. In many infected countries, this condition might not be feasible, owing to the cost of implementing a stamping-out policy, compensation and strong resistance from farmers against the adoption of such a policy. Control of CBPP has been carried out by immunization, imposition of movement control and, in some cases, the use of

[1] Animal Health Officer (Bacterial Diseases and Zoonoses), Food and Agriculture Organization of the United Nations, Rome, Italy

antibiotics although this is contrary to the OIE Animal Health code. This presentation is made to stimulate discussion on the subject of use or non-use of antibiotics and to indicate areas where research could be intensified to address the missing gaps in knowledge. It does not necessarily suggest a shift in official FAO policy on the use of antibiotics in treating CBPP disease.

THE DEBATE

The use of antibiotics to control CBPP still remains a controversial issue although farmers, paraveterinarians and veterinarians are prescribing the use of antibiotics in the field to control CBPP. This is even more pronounced because developmental policies tend to encourage the privatization of clinical veterinary-service delivery, resulting in promotion of antibiotics use as the shotgun approach to treating many respiratory infections (including CBPP) with drugs.

There is a school of thought which argues that antibiotic therapy could be a valid alternative to vaccination. According to this school of thought, the advantage of antibiotics over vaccines is that the drugs are already available on the market and their use may have a direct impact on poverty alleviation by diminishing the mortality rate of cattle, sometimes the sole property of the rural poor. Vaccines are distributed by state veterinary services exclusively and may fail to reach cattle owners who need them. Coupled with this, few countries have the requisite financial resources to mount yearly vaccination campaigns to control CBPP. On the other hand, arguments have been put forward by the school of thought that opposes the widespread use of antibiotics, such as the inevitable question of residues that may lead to the emergence of resistant bacteria in the environment and the possible long-term carrier state that is often attributed to CBPP-infected cattle treated with antibiotics.

For these reasons, veterinary services in countries in Africa affected by the disease have difficulties in defining an official strategy or policy concerning the use or non-use of antibiotics in CBPP disease management. This dichotomy of opinion is often observed in many CBPP meetings in which this subject matter has been discussed. Some directors of veterinary services hold that the use of antibiotics for the treatment of CBPP must be forbidden (although it is a very common practice in the field), whilst others consider their use as a possible tool to control the disease despite OIE Animal Health code stipulations to the contrary.

In the view of the author, the difficulty in the antibiotics/CBPP "controversy" is that there is paucity of data concerning the efficacy of antibiotic treatments *in vivo*. The lack of a suitable laboratory animal model for CBPP disease poses great technical difficulties in *in vivo* antimicrobial efficacy studies, similar to that observed in evaluations for CBPP vaccine efficacy studies. Many antibiotics have been shown to be active in *in vitro* assays against *Mmm*SC and this has been observed in the case of tetracyclines, macrolides, lincosamines, streptogramines and fluoroquinolones (Ayling *et al.*, 2000). Yaya *et al.* (2004) observed that antibiotic treatments using tetracycline in the field did not lead to the disappearance of the infection, but could reduce the infective pressure on susceptible cattle.

THE HYPOTHESIS

That antibiotics may encourage creation of a carrier status is at the centre of the dichotomy of opinions on the use of antibiotics to control CBPP. At the 3rd FAO/OIE/OAU Expert Panel meeting on CBPP in Khartoum in 1967, it was reported that "The mass drug or antibiotic

treatment of CBPP should be discouraged." Seven years later, Dr Alain Provost (1974) stated that spiramycin at a dosage rate of 25 mg/kg given in the early stages of infection causes regression of symptoms and bacteriological cure. He further stated that smaller doses are contra-indicated as they *may* create carriers. This hypothesis has still not been fully tested since the days of Provost. Carrier animals are thought to be a source of fresh infection in a herd and carriers are not easily detectable by clinical and serological tests. At the moment, there is little or no experimental evidence to support the hypothesis of carrier status created by the application of antibiotics. Studies are therefore urgently needed in this area to further elucidate the role of different classes of antibiotics in the generation or non-generation of carriers of CBPP disease.

Windsor and Masiga (1977) found that sequestras do not break down easily even following severe stress (such as deprivation of feed and water, injection of corticosteroids and splenectomy). Their conclusion was that sequestration was a host immunological reaction aimed at creating a barrier between the infective agent and its system. Hübschle *et al.* (2006) could not recover any *Mmm*SC from fibrotic sequestra after antibiotic administration. Thiaucourt (personal communication) could not isolate *Mmm*SC from sequestras of animals experimentally infected with *Mmm*SC and also found material in the sequestras to be sterile. Assuming that administration of antibiotics to treat CBPP caused the creation of sequestras, available empirical information suggests that these sequestra may be sterile and a break-up of sequestra may be regarded as a very rare event or may not even occur. Studies are urgently needed to test this hypothesis.

NEW CHEMOTHERAPEUTIC AGENTS

In the light of development of new and potent mycoplasmacidal drugs and other chemotherapeutic agents, it might be well revisiting the issue of antimicrobial therapy in the control of CBPP because it is a fact of life in the field. The international organizations (notably the OIE, FAO, AU-IBAR and others) require scientific data in order to revisit aspects of the Animal Health code that deal with this issue. Therefore, from a scientific and technical viewpoint, we owe it as a duty to provide an answer to the farmer considering the fact that at field level, no one appears to honour the edict that antibiotics should not be used in the treatment of CBPP disease. Proper guidelines or restrictions are required based on sound scientific data if antibiotics are to be used or not. Research in chemotherapy for CBPP should be pursued as a research option appropriate for the private sector–pharmaceutical industry/scientific community partnership.

THE CHALLENGES

The use of antibiotics for treatment of CBPP is forbidden, but farmers use them and are willing to pay anything to treat cattle acutely infected with CBPP. Obviously, some salutary effects have been observed by the farmers – which is why they continue to use antibiotics despite official sanctions. We should investigate thoroughly the therapeutic effects or otherwise of antimicrobial therapy so as to provide the relevant technical platform on which to include or exclude antibiotic treatment in the arsenals of tools to be used in the control of CBPP. Treating CBPP with antibiotics in Africa has almost always been a decision of the farmer who wants to limit the mortality rates of cattle infected with CBPP.

The author has had experience in the field where cattle infected with streptothricosis caused by *Dermatophilus congolense,* other bacterial infections and piroplasmosis, were treated with long-acting tetracycline against these diseases. Should the cattle be infected with CBPP, they would have inadvertently received doses of this drug which may have an effect on the course of CBPP disease including masking clinical signs, delaying early detection of the disease and possibly decreasing CBPP vaccination response. Provost and Queval (1968) demonstrated that a commonly used trypanocidal drug at that time – Mépacrine – was bacteriostatic *in vitro* against *Mmm*SC at 10 µg/ml. Yet it did not stop the process of development of Willem's reaction nor cure clinical pleuropneumonia *in vivo*.

The following gaps in our current knowledge of chemotherapeutic agents in modulating CBPP disease need to be clarified in addition to further understanding of the pathogenesis of the disease. The list could be further expanded during this consultation to provide research elements on the use of antibiotics for CBPP control:

- treatment of an infected CBPP herd leads to production of a larger number of chronic carriers (lungers);
- proportion of cattle with sequestra that harbour infectious *Mmm*SC and for how long;
- risk of CBPP disease transmission from chronic carriers;
- whether a single injection of a suitable preparation can result in sterile *Mmm*SC status, such as long-acting tetracycline do in contagious bovine pleuropneumonia (CCPP);
- effect of treatment on the immune response of cattle to *Mmm*SC infections and to vaccination;
- baseline information on antibiotic residues in milk and meat and the design of a monitoring system to ensure public safety and monitor the emergence of antibiotic resistance strains of *Mmm*SC;
- cost–benefit analysis for comparison with other control options or combinations thereof.

Recent initiatives from the ALive (Africa Livestock) platform in CBPP research, which include studies on antibiotic therapy of CBPP coupled with that previously commissioned by the Pan Africa Control of Epizootics (PACE), could provide further information and insights in addressing some of the gaps in knowledge on the efficacy of antibiotic therapy against CBPP. It must be recognized that the OIE Terrestrial Animal Health Code cannot be amended without science-based evidence on the efficacy of chemotherapeutic agents in the control of CBPP disease, which could provide another tool in the progressive control of the CBPP.

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Summary of Recommendations

PREAMBLE

The 4th Consultative Group on CBPP met from 6–8 November 2006 at the FAO Headquarters in Rome, Italy. Participants were welcomed by the Chief Veterinary Officer (CVO) of FAO and Chief of the Animal Health service Dr Joseph Domenech who called on the participants to come up with innovative ideas on how to deal with the current widespread use of antibiotics in treating CBPP in the field. The Director of Animal Health and Production, Dr Samuel Jutzi, opened the meeting and emphasized that FAO takes the control of animal transboundary animal diseases (TADs) seriously, especially in relation to CBPP. He informed the group that the recent establishment of the Crisis Management Centre (CMC) will not only concentrate on HPAI but also on other TADs such as CBPP. The Statement of the Purpose of the Consultative Group Meeting was read by Dr Juan Lubroth, Head of the EMPRES Infectious Diseases Group. The meeting was attended by about 28 participants, consisting primarily of representatives of FAO, OIE, AU-IBAR, IAEA, CVOs, heads of institutions involved in CBPP research and other scientists from Africa and Europe.

Technical presentation on the use or non-use of antibiotics, CBPP vaccines and molecular research, Diagnostics and surveillance and CBPP disease epidemiology and modelling were presented. Discussions emanating from these presentations were lively and educative. Two main working groups on the use or non-use of antibiotics based on different CBPP epidemiological scenarios and CBPP vaccine use and research were formed. The following recommendations were made by the group in relation to the theme of the meeting, the various presentations and discussions held. Progress made in antibiotic research at both laboratory and field was recognized and some of the recommendations made are derived from the observations made in these studies.

GENERAL RECOMMENDATIONS

1 The group recommended that scientific bodies engaged in CBPP research should periodically exchange research outcomes and ideas with the relevant stakeholders to effect the advancement of the control of CBPP in Africa.

2 The group endorsed the initiative of the ALive and other research initiatives on CBPP and requested donor agencies to consider its funding with respect to enhancement of CBPP surveillance systems, research into potent and thermotolerant vaccines and implementation of new integrated strategies.

ANTIBIOTIC USE IN CBPP CONTROL

3 In pursuing further studies (*in vivo* and by modelling) it was recommended to take the following parameters into consideration:

- The indications are encouraging but arguably not enough is known about the consequences of treatment:

- the impact of therapy on the subsequent immunity after recovery;
- the possibility that chemotherapy followed by vaccination in a herd could achieve complete elimination of infection.

• Further trials of the use of chemotherapeutic agents in CBPP control are needed:
- the effect of antimicrobial combinations with or without anti-inflammatory drugs in controlling clinical disease and infection;
- the efficacy *in vivo* of candidate antimicrobials against infection, transmission and overt disease.

• While conducting confined trials there should be extensive parallel studies to test improved control options combining chemotherapy, vaccination and zoosanitary procedures, in real situations.
• Operational strategies should include a consideration of the risks which could arise from antimicrobial use in control programmes.
• Effect of treating all animals in a herd should be compared to treatment only of clinical cases with or without subsequent vaccination.

4 On the application of antimicrobial use to various control scenarios it was recommended that antibiotic therapy be included with other options within holistic strategies which will take account of the need for:
• Surveillance including monitoring for lesions at abattoirs and slaughter slabs.
• Zoning according to farming systems, established human and livestock movement patterns and demographics, i.e. based on epidemiological analysis of the determinants of infection.
• Culling of affected herds with compensation where feasible ("stamping out").
• Cattle-movement management and animal identification with or without testing for infection.
• Vaccination of targeted to vulnerable groups and not limited to institutionalized, pulsed, annual, area-wide mass vaccination.
• Marking of vaccinates – ear tagging or other options.

5 It is recommended that the application of antibiotic use for CBPP control be considered within the following epidemiologically defined scenarios:
Scenario 1: a newly infected herd/area
• Ideally check diagnosis with a rapid pen-side test followed by laboratory examination.
• Stamping out by total slaughter of infected herds with compensation is best followed by monitoring of status.

If this cannot be achieved, then the "endemic infection" scenario would prevail:
Scenario 2: infection and disease in an endemic situation
Within the national strategy setting and under official veterinary service supervision test a package of provisions comprising:
• Confirmation of diagnosis by applying case definition/rapid test.
• Treat all cattle in the affected "herd" with a full course of appropriate antibiotics.
• Check diagnosis by sampling for laboratory testing.
• Vaccinate all cattle in "herd" after one month.
• Minimize livestock movement, even if only temporarily.

- Vaccinate all cattle in the vicinity, targeting an epidemiologically appropriate population.
- Monitor and, if necessary, re-treat affected herd.
- Revaccinate "herd" and then yearly thereafter for a defined period (an "exit" strategy is needed).

CBPP CONTROL VACCINES

6 Considering the task of maintaining the functional capacity of the Pan African Veterinary Vaccine Centre (PANVAC) in order to fulfil its mandate, it is recommended that resources for operational and technical activities are ensured.

7 Effective surveillance is a prerequisite for proper CBPP control. A defined epidemiological strategy should be supported by clinical-, abattoir- and laboratory-based surveillance. Laboratory and field capacity must therefore be strengthened to support surveillance- and CBPP-control activities. All available information on suspected/confirmed cases of CBPP must be made available at the national veterinary service to permit appropriate timely decision-making on CBPP control measures.

8 Public vaccination should be applied in a focused or targeted manner within the context of a defined epidemiological strategy. Examples are phased or roll-back programmes that channel resources to achieve specific goals in a time-bound programme are used. National mass vaccination programmes should be avoided as unsustainable and frequently unachievable.

9 Serious consideration should be given to the identification of alternative vaccines to those that are currently available. This will require a clear understanding of the pathogenesis of the disease and the mechanisms involved in immune protection. Substantial effort should be placed in defining these parameters so that progress can be made in development of improved vaccines. These efforts should draw widely on the broad capacities available within the CBPP research community.

10 In endemic settings, a national CBPP control programme should involve participation of the private sector. Wider access to vaccine should be through enhanced private-sector supply channels regulated by the national veterinary service. This is not intended to replace public-sector programmes, but instead to complement and support national control.

11 The culling of infected stock in a newly infected area is a preferred option to mass vaccination in controlling CBPP in a cost-effective way. At the same time, a surveillance zone should be established to protect the area.

12 Pilot studies to compare the impact of integrated control strategies in endemic areas should be undertaken. The efficacy of vaccination with current vaccines, treatment with pre-tested antibiotic regimen, and combined vaccination and treatment programmes should be determined without delay.

13 The density of abattoirs/slaughter slabs/slaughter points needs to be increased in relation to markets and surveillance needs to be carried out in order to reduce the need for trekking animals, thus reducing the risk of spreading infection.

Dated: 8 November, 2006

Summaries of presentations and discussions

CBPP CONTROL: ANTIMICROBIAL RESEARCH

Summary

Dr Mamadou Niang introduced contagious bovine pleuropneumonia (CBPP) as a priority disease in Africa. In spite of vast vaccination campaigns in some African countries, the disease persists and poses the problem of immediate alternative solutions (i.e. rigorous restriction of cattle movement, slaughter and compensation) for controlling the disease. The objective of the study reported was to evaluate the ability of long-acting oxytetracycline to clinically and bacteriologically cure CBPP-infected cattle and to determine the risk of transmission of the disease from these treated animals. Based on the clinical evidence, a high rate of recovery was obtained (10/12) following treatment with the long-acting oxytetracycline. Nevertheless, all 12 animals showed a persistent seroconversion throughout the entire experimental period, and the presence of chronic lesions were noted at necropsy. These were characterized by pulmonary adherence in all the 12 animals and visible pulmonary sequestra in 4 of them. *Mmm*SC was isolated in pure culture from the sequestral contents taken from these animals. In contrast, healthy animals put in contact with these 12 animals remained clinically normal throughout all the experimental period and, at necropsy, no pathological changes characteristic of CBPP were noted; laboratory analysis was also negative. These results can have important implications in the control strategies of the disease in African conditions.

Dr Robin Nicholas explained the findings of a recent study on the effectiveness of danofloxacilin (Advocin) treatment. Large reductions were seen in mortality and morbidity rates in 13 CBPP-affected herds treated with Advocin (2.5 percent) with no deaths due to CBPP being seen between six and nine months. The number of seropositive animals increased in herds three months after treatment but declined after nine months. Generally, mean serological titres were reduced over the nine months from 1/80–1/160 to 1/10–1/20. Only six new seropositive cattle were seen after nine months

Dr Jeff Mariner pointed out that there was no published scientific evidence of new infections after a breakdown of sequestra and of the establishment of carriers of CBPP after antibiotic treatments. According to his mathematical model of CBPP transmission, most new infections were from actually diseased animals and new introductions of *Mmm*SC could result in the circulation of the contagion and disease for six years if unchecked. Quarantine and movements control resulted in a reduction in disease incidence. Vaccination alone at 70–80 percent effectiveness would never eradicate the disease, but would reduce mortality. A reduction in transmission by one-quarter would result in near eradication of the agent from that population.

Dr Roger Ayling reported the results of a study of the sensitivities of a several *Mmm*SC strains to a variety of antibiotics. *Mmm*SC was susceptible to many antimicrobial agents *in*

vitro and strains that were isolated from very different geographical locations varied little in their susceptibilities.

Discussion

The Chair reminded the group that previous meetings have always concluded with more or less similar findings and that there has always been the need for more scientific work. He stressed that there was a unique opportunity at this meeting, especially after hearing the excellent overview on antibiotic therapy, of making strong and different recommendations that tackled the problems in the real world. He opened the forum for discussion.

Dr Francois Thiaucourt, commenting on the work of Dr Niang, explained that antibiotics reduced the clinical symptoms and the post-mortem lesions of CBPP; the lesions (not sequestra) contained *Mmm*SC. He suggested that treatment would reduce losses but that it was not a comprehensive cure. There were no data on carrier animals, on how contagious they were, or on the actual transmission or risk of transmission they pose. The assessment of this risk was very important especially for disease-free countries. In the studies reported by Dr Niang, there was a reduction in transmission. Nevertheless, there was some transmission. Discussions that followed resulted in establishing that the reduction of transmission was very important and that the utility in the detection of contagion in an affected population was to estimate the extent of transmission.

It was noted by Dr Mariner that the reduction in transmission because of antibiotic treatment was remarkable and Dr Roeder proposed that a combination of vaccination and antibiotic treatments may lead to better control, but due considerations must be given to differing disease scenarios when considering intervention strategies (for example, new introduction in free country, epidemic resurgence in endemic situations, and endemic disease). Models tested mass effects and not individual reactions: therefore, reductions in disease incidence (say due to antibiotic therapy) did not involve necessarily the treatment of all animals. In Tanzania mass vaccination campaigns were of dubious impact because of the inconsistency of application and the Massai usually turned to antibiotic therapy. The spread of CBPP in all cases was attributed to the failure to implement minimum veterinary services.

Control campaigns either with roll-back vaccination or antibiotics would be difficult to establish and maintain and treatment regimes would need to be clear. In the Namibian experience, there were still odd cases of CBPP even after ten years of vaccination. There are no reliable laboratory test systems to monitor vaccination effectiveness; regular post-mortem examinations are not feasible; and abattoir surveillance (used for compliance of the OIE pathway) is inadequate because not all cattle are slaughtered at the abattoir.

The Chair concluded that antibiotics may be a useful tool against CBPP but that the regimens, conditions for use and withdrawal period required careful considerations, and posed questions regarding funding sources, cost recovery, and effectiveness compared with proper vaccination.

CBPP VACCINES/MOLECULAR RESEARCH

Summary

A multilocus sequence typing (MLST) scheme applied to *Mmm*SC isolates was presented by Dr Thiaucourt. MLST was capable of fine differentiation between strains of *Mmm*SC and

has shown that recent CBPP outbreaks in Europe were due to resurgence of the disease and not because of reintroduction of the agent. In Tanzania there was an introduction followed by clonal expansion.

Dr Joseph Litamoi presented results of a preliminary study on the usefulness of the xerovac process as a method for preparing relatively heat-resistant live attenuated CBPP vaccine. CBPP vaccine cultured in growth medium with or without trehalose was dehydrated in an excipient containing different strengths of trehalose concentrations and tested for heat tolerance. It was shown that dehydration of the vaccine in a stabilizer containing a high concentration of trehalose conferred additional heat resistance to the product compared to a similar preparation made using a short or extended lyophilization cycle. Subject to further experimentation, the use of the xerovac process could be an alternative method of increasing the heat tolerance of dehydrated CBPP vaccine compared to similar products prepared, using established procedures such as freeze drying.

Dr Schneir provided an overview of a collaborative research project developed in 2003, involving the Moredun Research Institute (UK), the University of Bern (Switzerland), the International Livestock Research Institute (Kenya), the Kenya Agricultural Research Institute, the Animal Diseases Research Institute (Tanzania), *Vétérinaires Sans Frontières* (VSF) Germany and the Kenya Department of Veterinary Services. The efficacy of buffered vaccine (trialled in the Massai ecosystem), socio-economic aspects, vaccine-distribution systems, evaluation of diagnostic tests (LppQ-ELISA, latex-agglutination tests for antigen detection, real-time TaqMan PCR), the immunology of vaccination and new generation vaccines (genetically modified T1/44) were the tasks for study in this project.

Dr Otto Hübschle's observation that the immunosuppressive agent Cyclosporine delaying the events that follow infection supported further the important role of the immune response on pathogenesis of CBPP.

Discussion

The MLST technique was a PCR/sequencing-based technique and could not be modified to produce a simple, single reaction PCR test for epidemiological use, and it did not test virulence factors.

The expense of modifications to the existing vaccine that may involve the use of trehalose was questioned. Fortunately, trehalose that used to be expensive is now the same price as sucrose and will not add a significant cost to the product. A comment that it may not work with CBPP vaccines was unfounded and not substantiated.

There was discussion on the appropriate diluents for the existing vaccine. The unsuitability of $MgSO_4$ in the reconstitution buffer because it caused a pH change leading to catastrophic decrease in viability was stressed. Phosphate-buffered saline was the preferred diluent. Saline diluent was used recently in Zambia and did not result in a drop in titre of vaccine.

Some likely explanations for the inconsistency of occurrence of post-vaccinal reactions were offered. These adverse reactions were seen most often in naïve populations; thus, they may not have been seen in Massai cattle and in other populations that have been previously primed. The causes or components responsible for these reactions were not known. Other factors such as vaccination technique also played a part in post-vaccinal reactions.

CBPP DIAGNOSTICS/SURVEILLANCE

Summary

Dr Herman Unger described the key objectives of the IAEA-coordinated research programme. He mentioned that it was to assist national veterinary laboratories in the diagnosis of CBPP and in the monitoring of national and regional CBPP-control programmes. Dr Unger explained the progress of the tasks under the Coordinated Research Programme (CRP). These were: i) evaluation of the serological tests for fitness for purpose (c-ELISA, LPPQ-ELISA); ii) introduction of isothermic PCR (LAMP) for the early and specific detection of CBPP; iii) evaluation of tools and genetic markers for molecular epidemiology of CBPP and their potential use in control programmes; iv) evaluation of skin testing for the detection of infected and carrier animals (expression antigen, LPPQ).

Dr El Sawalhy provided an overview of the CBPP situation in Africa and stated that the estimated annual direct cost of CBPP infection was about US$2 billion. In 2003, 14 African countries reported 272 outbreaks; in 2004 20 countries reported 314 outbreaks; and in 2005 18 countries reported 156 outbreaks. AU-IBAR had commissioned a study into the use of antibiotics for CBPP and the report would be available shortly.

An overview of the evolution of CBPP in southern Africa was provided by Dr Musisi. Dr Belemu followed with an account of CBPP control in Zambia, which was followed by Dr Njau's account of the same in Tanzania. There have been several success stories in the control of CBPP in southern Africa. Angola is gradually rebuilding its veterinary infrastructure – the Central Veterinary Laboratory in Luanda and several mobile laboratories are active in the control of CBPP. In August 2003 illegal movements of cattle resulted in outbreaks of CBPP in the Caprivi strip of Namibia about 30 km from the Botswana border and 10 km from the Zambian border. The successful control strategy in eastern Caprivi was vaccination and movement control with good communications between the authorities and communities in the three countries. Many regions of Tanzania were now also clear of CBPP. The general problem that follows is managing low-prevalence CBPP combined with with limited resources, unknown socio-economic impact, lack of movement control and insufficient scientific tools to support the claim of freedom from infection and disease.

CBPP spread in three fronts in Zambia; to the north-west, eastwards and south-westwards. For a time, the disease spread unrecognized because of the reorganization of veterinary services and when flooding hampered vaccination delivery. The strategy of testing and slaughtering of suspected or infected cattle at the abattoir (and prompt compensation) quickly brought the disease under control. At the same time, public awareness programmes on the disease, some that targeted illegal cattle movements and others that explained the utility of cattle identification, were conducted. There was also effective assessment of disease-control programmes through better surveillance. In 2006 floods were again responsible for mass movements of people and cattle and disruptions in veterinary services that led to a confirmed outbreak of CBPP.

Tanzania has spent a large amount of resources towards the control of CBPP – in capacity-building in diagnostic infrastructure (laboratory), human resource development (training), mass vaccinations and surveillance countrywide. FAO has contributed towards the government efforts by supporting the interventions. Since 2003, a roll-back plan was begun and coordinated vaccinations carried out, starting from the Southern Highlands

zone moving northwards. In the roll-back plan cattle in each zone are vaccinated three times in the first year followed by annual vaccination for another four years, after which the zone becomes a surveillance area with vaccinations only being carried out depending on the outcome of active disease search. Preliminary results of the evaluation of the plan indicate that the disease prevalence has been reduced by 50 percent.

Overviews of the CBPP component of the African Livestock (ALive) research proposals were presented by Dr Thiaucourt, and control experiences of CBPP eradication in Portugal by Dr Botelho. ALive strives to enhance market access for African livestock through better control or eradication of animal diseases. The project proposes to work on vaccines, companion diagnostic tests and decision-support tools towards the elimination of CBPP from African livestock. Eradication of CBPP from Portugal was achieved with targeted slaughter of infected herds. Before 1988 all complement fixation test (CFT) positive herds were slaughtered. Thereafter, a system of serological surveillance using CFT following confirmation of positive samples with immunoblotting was used to identify "true" positive herds which were consequently eliminated, thus reducing the overall number of animals slaughtered. Post-mortem isolation of *Mmm*SC was always used to confirm immonoblot positive cases.

Discussion

The choice of using lipolysaccharide Q (LppQ) for skin testing was questioned. It became evident that LppQ was almost the only recombinant *Mmm*SC molecule available in a purified form. Reservations concerning its use were that it may not be an accurate measure of cell mediated immunity (CMI): rather, one would expect to see a CD4 response to LppQ alone. Roger Windsor tried whole Ag skin test without success. It was conjectured that both CMI and humoral immunity were involved in clearing *Mmm*SC but the components of the organism that elicit these were not known. Current antibody tests do not correlate with immunity/protection: thus, whatever they might measure cannot be the protective antigen(s). Lipopolysaccharide (LPS) contamination was also identified as a potential problem that might lead to a lack of sensitivity of skin tests. The utility of molecular epidemiology tools that were not developed in Africa were also questioned because most African laboratories did not have the capacity to carry out these tests.

In Portugal, abattoir surveillance was used and suspicious lesions were tested in the laboratory. "A lot" of false positive samples were found with CFT, and IBT was more specific, but they were always followed up with isolation because IBT may also show some false positive results. C-ELISA technique was developed because there were problems with CFT in relation to specificity. However, c-Elisa also has problems: it can only diagnose CBPP accurately when it is positive for an extended period. CFT that detects IgM and maybe IgG1 is positive first during infection and has a duration of several months. c-ELISA positive antibodies appear shortly afterwards and persist maybe for years and their appearance apparently correlates with lesion formation. c-ELISA has now been standardized and so comparisons between laboratories can be carried out to provide conclusive results on how well it works.

Strong recommendations in Accra (2003) and Conakry (2004) were that mass vaccination was the way forward, but this was difficult to implement. The AU-IBAR plan has

not been achieved because there was no strategy to overcome the shortcomings on the ground. In addition, there were no open plans regarding movement control, abattoir trace-back and how to tackle the problem at source. It was generally felt that there was little advancement in the knowledge of CBPP and not enough serious information had been gathered to attract serious funding from national governments and donors. However, under AU-IBAR two trials were carried out: on (i) on vaccine use and (ii) on antibiotic use in Ethiopia, but reports are still unavailable. Vaccine was maintained in the public sector – again, there were insufficient funds to ensure proper vaccine coverage. There was a need to enhance access through the private sector. PANVAC still existed and continues to operate. All vaccine batches should be checked by PANVAC but this is not always done.

It was noted that, overall, a regional approach was missing from CBPP-control efforts. There was close dialogue between Zimbabwe and Zambia and there were some meetings in the Southern African Development Community (SADC) to streamline control of TADs. North Zambia, south Tanzania and Malawi interacted at the CVO level.

FAO's priorities also came under question and HPAI was a major concern at the moment but priorities are driven by government requests so ultimately the individual countries' priorities are reflected in FAO's actions. Under the impetus of HPAI there has been a great deal of focus on animal health and this has led to attempts to upgrade veterinary services in many countries.

Individual presentations

Notes:

- CBPP: AU-IBAR Perspective presented by A.A. El Sawalhy of AU-IBAR, Nairobi, Kenya. The paper was not submitted for publication in these proceedings.
- Papers are published as presented by the authors, in conformity with FAO requirements.

Presentation 1

Effect of antibiotic therapy on the pathogenesis of CBPP

Experimental transmission of the disease by contact from infected animals treated with oxytetracycline

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INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an important infectious disease of cattle that is characterized by a severe fibrinous exudative pleuropneumonia caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC). It used to belong to the OIE list of notifiable diseases because of its high potential for contagiousness irrespective of national borders. Now that the rinderpest is largely controlled in Africa, CBPP remains the most important disease in tropical Africa causing great economic losses including mortality, loss of weight, reduced working ability, reduced fertility and indirect costs due to control programmes.

At one time or another, in history, the disease has occurred in Europe, Asia and America. However, through a policy of rigorous restriction of cattle movement, slaughter and compensation, the disease has been eradicated on most parts of these continents. Due to socio-cultural and economical reasons, applications of such measures appear not to be feasible in most African countries. For these reasons, it is believed that, at this point in time, the only realistic way of controlling CBPP in many countries affected by CBPP is by massive and repeated vaccination against the disease.

In Africa, attempts to control CBPP by a combination of mandatory vaccination along with quarantine of affected animals dates back to the colonial period. However, there is some evidence that so far this conventional approach has not been successful in eradicating CBPP, because the incidence of the disease continues to increase in an alarming way in several parts of the continent. It seems that the current vaccination campaigns serve only, at least for the time being, to suppress clinical disease to manageable proportions but

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not eradicate infection. Furthermore, quarantine is impossible to enforce for an extended period of time because of the conditions of extensive communal grazing and transhumance which are prevailing in most African countries.

Alternative means of controlling the disease including antibiotic treatment have always been discouraged without receiving any scientific support. In fact, it is a common belief that treatment of infected animals with antibiotics compromised the control of the disease by generating a large number of carrier animals (FAO, 1967). Such an ingrained understanding by the scientific world prevented the possibility of using antibiotic treatment as an option in the control of the disease. Looking into the literature, it seems, however, that this perception has not been objectively assessed and, indeed, there are old reports indicating the contrary (Moret *et al.*, 1949; Moret *et al.*, 1951; Turner, 1960; Hudson and Etheridge, 1965; Provost, 1974; Windsor, 1977). More than that, illegal treatment of the disease with antibiotics by livestock owners and even by veterinary auxiliaries is a frequent event in African countries. Indeed, such an illegal practice, despite official condemnation, will surely increase as the privatization of clinical services and the availability of antibiotics increase. Recently, the issue was largely debated during the FAO electronic conference (FAO, 2002) and the AU/IBAR meeting in Accra, Ghana (2002). It was indicated that, although it is suitable to prohibit the use of antibiotics for treatment of CBPP because of the perception that it may predispose infected animals to become chronic carriers, there is still very little scientific information to substantiate such a perception and also the practice is very common in the field.

In the light of the above, we think that what is required now is not the categorical condemnation of the usage of antibiotics in the treatment of the disease but efforts to optimize drug formulation and regimens for use to meet established and accepted criteria for its control. Therefore, we think that there is a pressing need to objectively evaluate the therapeutic value of the numerous available chemotherapeutic agents such as tetracycline, tylosin, spiramycin and many other new antibacterial agents in the treatment of CBPP.

To address some of these needs, a research project was developed. The project's main objectives were to gather evidence, through animal experimentations, and to support or disprove the perception that the use of antibiotics may cause infected animals to become chronic carriers which may become potential sources of spread of the disease by assessing: (i) the efficacy of the use of antibiotic treatment against CBPP; (ii) the role of antibiotic treatment in the development of sequestra (chronic carriers) in CBPP-infected animals; and (iii) the risk of transmission of the disease from animals with sequestra or infected animals treated with antibiotics. It is expected that a better understanding of these aspects could contribute to the possibility of antibiotic treatment being incorporated in to control strategies of the disease in African conditions.

This report presents preliminary results obtained from a trial aiming to assess the efficacy of long-acting oxytetracycline (LA oxytetracycline) to treat CBPP-infected animals and to determine the risk of transmission of the disease from these treated animals.

MATERIALS AND METHODS

Animals and experimental design

Twenty clinically healthy zebu cattle, three to six years old, were obtained from several herds in a relatively CBPP-free zone and conveyed to our facilities in CVL, Bamako. The

cattle in these herds had at no time been exposed to CBPP infection and had not been vaccinated against the disease during the past ten years. This was based on the data given by the local animal health services (DNSV-Mali, 2004) and the information given by the owners. The selected animals were negative for antibodies to *Mmm*SC as attested by slide agglutination test, complement fixation test (CFT) and competitive enzyme linked-immunosorbant test (c-ELISA). They were also free from brucellosis and tuberculosis as judged by slide agglutination and skin tests, respectively. Upon arrival, the animals underwent several months' quarantine during which they were bled at a regular basis for confirmation of their serological status, treated against various parasites and vaccinated against pasteurellosis and blackleg.

For procurement of CBPP-infected animals, 15 sick animals (*N'dama*) were collected in the field during an active CBPP outbreak. The confirmation of the outbreak was made by clinical, pathological and microbiological findings. Selected animals were treated with LA oxytetracycline (20 percent) following the manufacturer's instructions (dose rate of 1 ml/10 kg of body weight on Day 1 and Day 3) before being transported to the CVL facilities.

After one month of observation, treated animals were introduced to the healthy animals. Animals were tagged with numbers from C1 to C20 for the contact-exposed animals and I1 to I15 for the CBPP-infected and treated animals. To increase the pressure of infection, the infected animals and the contact-exposed animals were kept in permanent and close contact in an isolated and secured pen. They spent the day time in the courtyard of the pen with free access to food and water, and the night time in containment within the pen.

Clinical and post-mortem examinations

In order to determine the transmission of the disease, animals were maintained up to 10 months after contact and monitored daily for clinical signs of illness including cough, nasal discharge, lethargy, respiratory distress, anorexia, weight loss and eye-watering. In addition, rectal temperatures and respiratory pools were recorded daily throughout the duration of the experimentation.

Complete necropsies were performed on animals which died during the experimentation or which were slaughtered at the end of the experimentation. Lungs were thoroughly examined for gross pathological lesions of CBPP and findings were recorded in detail. In addition, other organs, including heart, kidneys and digestive tract, were examined in detail.

Sample collection

Blood for sera, broncho-alveolar lavage fluid (BAL) and nasal swabs were collected concurrently in sequence at one-to two-week intervals during the experimentation. Samples of lung tissues, pleural liquid, lung sequestra, lymph nodes and kidneys were also collected from animals which died during the experiment or were slaughtered at the end of the experimentation. Blood for serum samples were allowed to clot at room temperature and then centrifuged to harvest the serum samples that were aliquoted and stored at –20°C until tested for antibodies (Ab) to *Mmm*SC. Samples of BAL, lung tissues, pleural liquid, lung sequestra, lymph nodes and kidneys were processed immediately for mycoplasma isolation.

Laboratory analysis

For the isolation of *Mmm*SC, ten-fold serial dilutions of each sample were made in Gourlay and brain heart infusion (BHI) broth media. Confirmation of *Mmm*SC was made by immuno-fluorescence test after two to three sub-passages on solid medium.

To test specific antibodies to *Mmm*SC in the serum samples, complement fixation test (CFT) and competitive enzyme linked-immunosorbant test (c-ELISA) were both used according to the protocols provided with the kits (CIRAD-EMVT, Montpellier, France).

Results

Four healthy cattle (C6, C10, C13 and C19) died shortly after arrival with no obvious clinical signs of infection. At necropsy, no pathological changes characteristic of CBPP infection were noted. In addition, one infected animal (I12) died during the transportation from the field to the CVL. At necropsy, lesions characteristic of CBPP (lung hepatization) were noted. Therefore, data collected from the remaining 14 treated animals and 16 contact-exposed animals were utilized, here, in reporting results.

Clinical observations

All the contact-exposed animals remained clinically normal during the entire experimental period. As for the CBPP-infected and treated animals, the main clinical signs at their arrival were dominated by cough, prostration, dyspnoea, nasal discharge and weakness. All these clinical signs completely disappeared in all the animals (except two) within one to two months following the treatment with the LA oxytetracycline. Cough was persistent in these two animals and one died thereafter. The post-mortem diagnosis revealed the presence of chronic lesions characterized by big sequestra and *Mmm*SC was isolated from the contents of the sequestra.

Gross lesion findings

The results are summarized in Table 1.1. Contact-exposed animals showed no pathological changes characteristic of CBPP infection. In contrast, all the CBPP-infected and treated animals showed chronic lesions characterized by lung adherence in all of the 12 animals and visible pulmonary sequestra in 4 of them (I1, I4, I10 and I14).

Serological responses

The results of the serological follow-up are presented in Figures 1.1A, 1.1B, 1.2A and 1.2B. Throughout the whole experimental period, none of the contact-exposed animals developed Ab titres to CBPP in both CF and c-ELISA tests. Conversely, sustained seroconversion with high titres in both c-ELISA and CFT were observed in all CBPP-infected and treated animals.

Mycoplasma isolation

It can be seen from Table 1.2 that *Mmm*SC was isolated only from the sequestral contents of the lungs of four CBPP-infected and treated animals (I1, I4, I10 and I14). Attempts to isolate mycoplasma from the samples of BAL, lung tissues, lymph nodes and kidneys collected from both groups of animals were unsuccessful.

TABLE 1.1
Results of lesions findings

CBPP-infected and treated					Contact-exposed				
No.	Lung adherence	Sequestra	Hepatization	Pleural fluid	No.	Lung adherence	Sequestra	Hepatization	Pleural fluid
I1	+	+	–	–	C1	–	–	–	–
I2	+	–	–	–	C2	–	–	–	–
I3	+	–	–	–	C3	–	–	–	–
I4	+	+	–	–	C4	–	–	–	–
I5	+	–	–	–	C5	–	–	–	–
I6	+	–	–	–	C7	–	–	–	–
I7	+	–	–	–	C8	–	–	–	–
I8	+	–	–	–	C9	–	–	–	–
I9	+	–	–	–	C11	–	–	–	–
I10	+	+	–	–	C12	–	–	–	–
I11	+	–	–	–	C14	–	–	–	–
I13	+	–	–	–	C15	–	–	–	–
I14	+	+	–	–	C16	–	–	–	–
I15	+	–	–	–	C17	–	–	–	–
					C18	–	–	–	–
					C20	–	–	–	–

Discussion

The present animal experimentation was designed to assess the efficacy of LA oxytetracycline to treat CBPP-infected animals and to determine the risk of transmission of the disease from these treated animals. The observations reported here clearly indicate that LA oxytetracycline treatment had a positive effect on the clinical course of the disease since the majority of the treated animals (12/14) recovered completely within one to two months following treatment. Nevertheless, the treatment did not allow the bacteriological cure of the infected animals since *Mmm*SC was recovered from the sequestral contents of the animals. Despite this, the disease was not transmitted to the contact-exposed animals in spite of a prolonged contact with the CBPP-infected and treated animals. Overall, the results obtained in this experimentation fit with the observations made by Windsor and Masiga (1977) and Yaya (2003).

ACKNOWLEDGEMENTS

This work was supported by the World Bank through the Government of Mali and the Pan African Control of Epizootics (PACE-Mali).

FIGURE 1.1A
Results of c-Elisa in CBPP-infected and treated animals

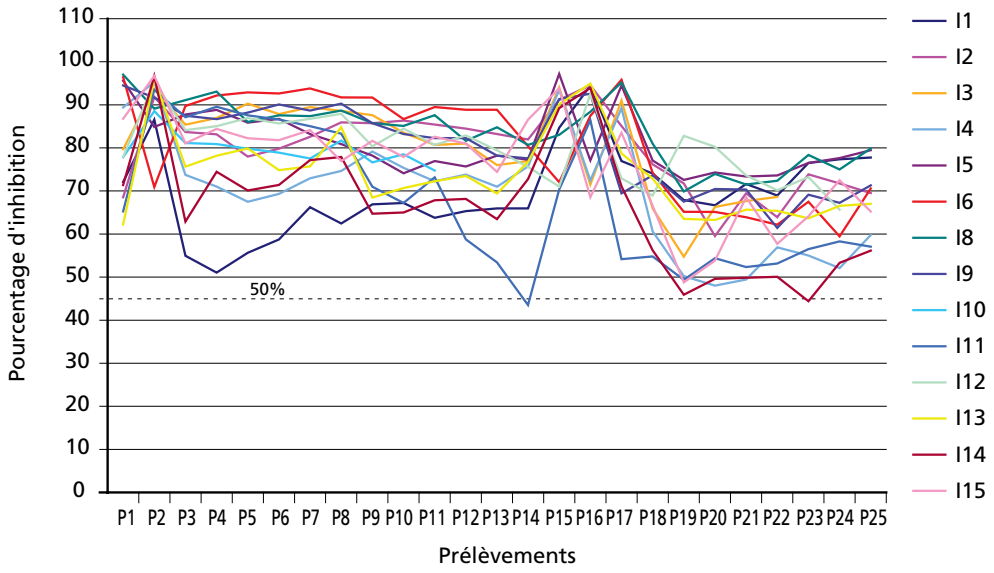

FIGURE 1.1B
Results of c-Elisa in contact-exposed animals

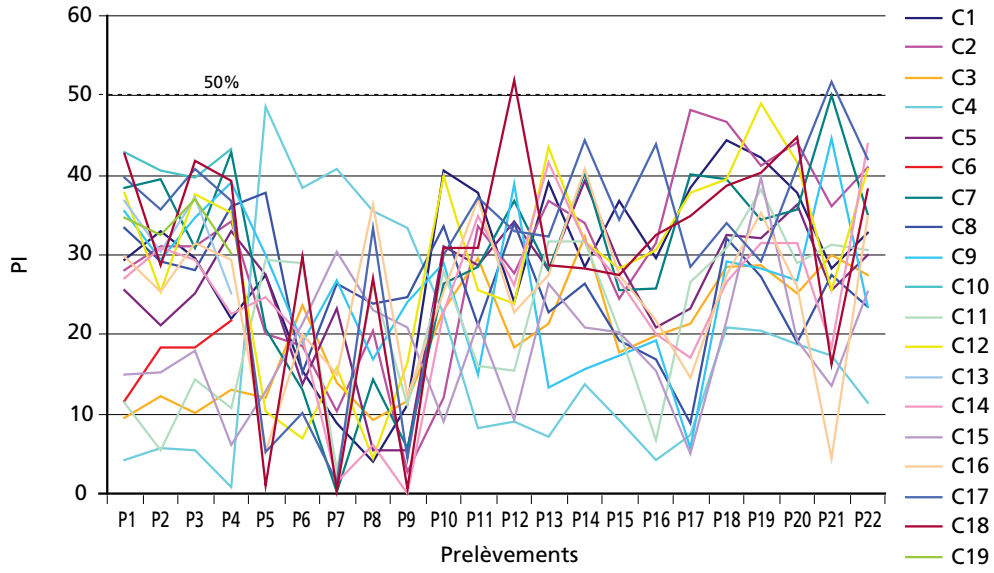

FIGURE 1.2A
Results of CFT in CBPP-infected and treated animals

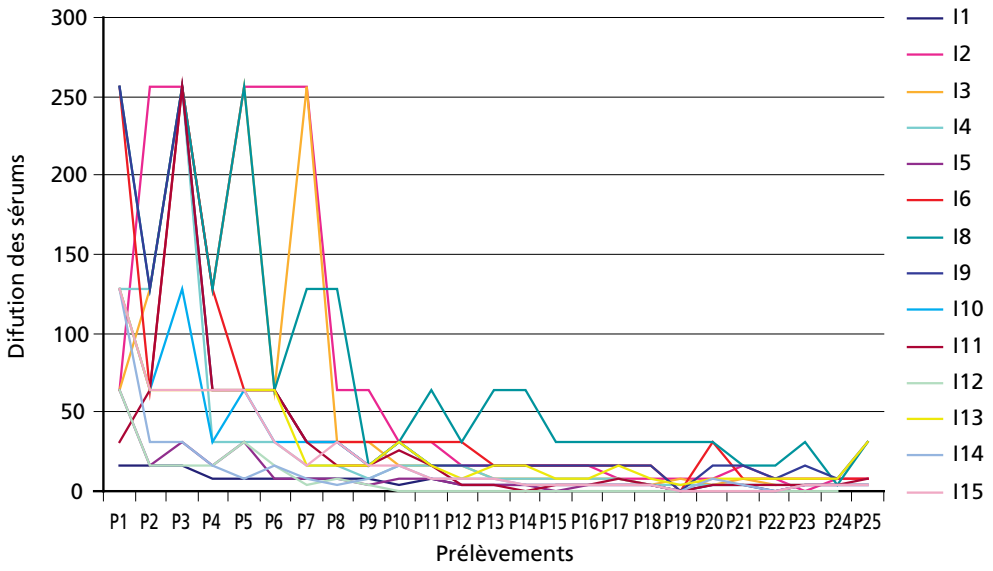

FIGURE 1.2B
Results of CFT in contact-exposed animals

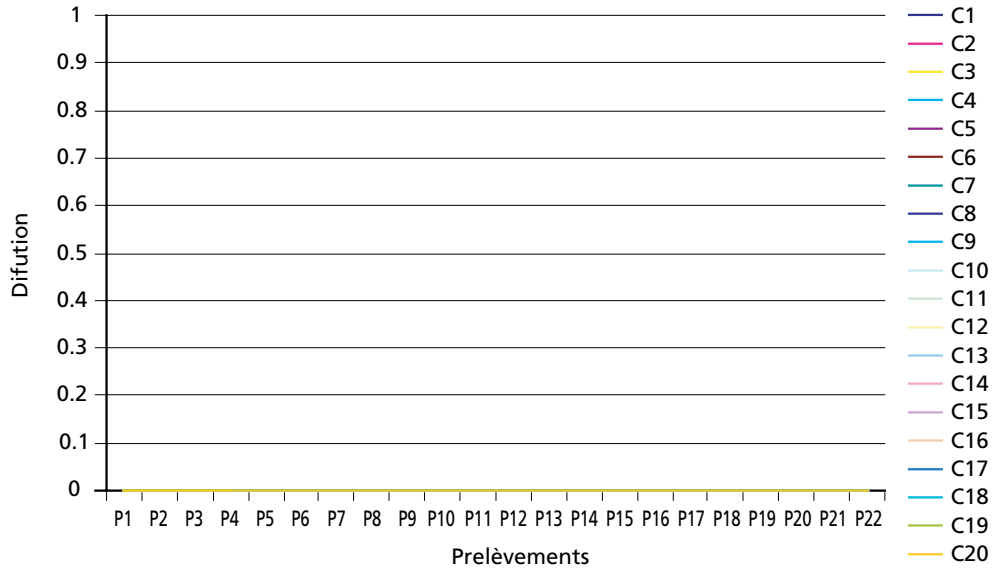

TABLE 1.2
Results of *Mmm*SC isolation

CBPP-infected and treated animals					Contact-exposed animals			
Animals	Lung	L. Node	Sequestra	BAL	Animals	Lung	L. Node	BAL
I1	–	–	+	–	C1	–	–	–
I2	–	–	–	–	C2	–	–	–
I3	–	–	–	–	C3	–	–	–
I4	–	–	+	–	C4	–	–	–
I5	–	–	–	–	C5	–	–	–
I6	–	–	–	–	C7	–	–	–
I8	–	–	–	–	C8	–	–	–
I9	–	–	–	–	C9	–	–	–
I10	–	–	+	–	C11	–	–	–
I11	–	–	–	–	C12	–	–	–
I13	–	–	–	–	C14	–	–	–
I14	–	–	+	–	C15	–	–	–
I15	–	–	–	–	C16	–	–	–
					C17	–	–	–
					C18	–	–	–

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Presentation 2

Effect of Advocin on the elimination of CBPP from the Caprivi region of Namibia

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INTRODUCTION

Cattle in the Caprivi region of Namibia became infected with CBPP in August 2003 for the first time in over 60 years, almost certainly as a result of cattle introduction from Zambia. Up to the beginning of the trial over 400 cattle had died as a result of CBPP and over 75 percent of farmers have had CBPP cases in their herds with nearly all the large-herd farmers experiencing cases. It was estimated by local veterinarians that many cattle remain infected putting at risk up to 44 small (7), medium (29) and large (8) herds totaling 40,000 cattle mainly in the Linyanti area comprising mainly the Maunga, Mbilajwe and Batubaja villages. Evidence suggested that vaccination was not controlling the outbreaks and indeed was leading to the production of severe adverse reactions and death which discouraged owners to vaccinate.

Preliminary trials at Mashere in 2004 had shown that the use of Advocin "off label" could greatly reduce the transmission of *M. mycoides* subsp. *mycoides* SC (*Mmm*SC) from infected to contact animals (Hübschle *et al.,* 2004). The study justification arose from the need to test the efficacy of the product in the control of CBPP when tested under typical local commercial animal management and disease conditions.

The aim of this project was to attempt to eliminate CBPP from a defined and controlled region of Namibia by identifying all affected herds and treating all cattle within these herds, including seronegative cattle, with danofloxacin (2.5 percent Advocin; Pfizer).

MATERIALS AND METHODS

Test materials

Compound: Danofloxacin (Advocin 2.5 percent).

Animals

Species: Local Namibian cattle breeds.

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[2] Consultant
[3] IZS Sicily
[4] Pfizer Animal Health
[5] CVL (Windhoek), Namibia

Experimental design

An experienced veterinarian (OA) was located near the area where the outbreaks were occurring to get a better understanding of the prevalence of CBPP in the region, mainly as a result of obtaining local veterinary knowledge and examining herds for clinical signs. This area was a 30-km region around Maunga, Linyanti, Mbilajwe and Batubaja, close to the Botswana border in which movement is restricted – no cattle can be moved in or out of the region (Figure 2.1). Cattle going to slaughter must be transported directly to the abattoir by vehicle. OA gained an understanding of local cattle movements in order to assess quantitatively the cattle population at threat. It was important that baseline disease data were obtained within this specific region to enable comparisons to be made on disease prevalence before and after treatment.

Sera were taken from 10 percent or no less than 3 in animals in 129 herds in the infected region. Herd sizes varied from 12–160 animals.

Once identified, all herds containing clinically and serologically positive cattle were treated with Advocin as recommended. OA carefully monitored the treated herds and actively investigated new outbreaks in at-risk herds including visits to abattoirs. Cattle owners were encouraged to have dying cattle examined post-mortem or to send lungs to the local veterinary centre to look for signs of CBPP.

At three, six and nine months post-treatment, herds were revisited for clinical and serological testing to determine success of project. Data on the prevalence of disease in the region were compared over this period.

In addition, 15 cattle from the infected region were purchased and examined post-mortem at the end of the study to determine the relationship between serological status and lesions. They fell into the following categories:

- Five which remained seropositive for the entire period of observation.
- Five which became seropositive after treatment.
- Five which become seronegative after treatment.

FIGURE 2.1
Study area believed to contain all CBPP affected animals indicated by circle

Serological and microbiological testing

CFT and PCR where necessary were carried out at the CVL, Windhoek.

Treatment administration

Each cow was treated intramuscularly with 2.5 mg/kg danofloxacin (2.5 percent Advocin, Pfizer) on three consecutive days. The test article was be supplied by Pfizer.

Data analysis

Morbidity and mortality rates at the beginning of the trial were compared with that obtained at three, six and nine months. Similar comparisons were made with serological data.

Results

Outset of trial

Prior to treatment 15 of 129 herds were identified as containing seropositive animals; of which 64 cattle were seropositive by CFT; over 50 animals were coughing. In total 663 cattle from the 15 herds were treated with Advocin. Average CFT titres were between 1/80 and 1/160 and occasional titres of 1/320. However, it was only possible to obtain complete data from 13 of the 15 herds.

Three-month examination

Three months after treatment of herds with CBPP-seropositive cattle, herds were revisited, examined for clinical signs and rebled. Sera were tested by CFT at the CVL Windhoek. During the 3 months following treatment, only three animals had died and nine were coughing. However, 153 new seropositive cattle were detected; although, other than the nine which were coughing, none were showing clinical signs. This suggested that infection was still present in the herds (i.e. the treatment had not completely sterilized the animals) though it is possible that immunity as seen by this antibody response was developing. Additionally, all previously seropositive cattle remained positive. CFT titres were generally low and variable with average titres between 1/20 and 1/80 and occasional titres of 1/160.

Six-month examination

All cattle were rebled after a further 3 months. Only one animal had died of CBPP during the second period. Overt clinical signs were absent. Compared to 153 new infections in the first 3 months, only 49 new infections were detected so while there were a total of 210 seropositive cattle detected at 3 months there were only 142 after 6 months. In all, 110 previously seropositive cattle had no detectable titre. CFT titres were generally low and variable with average titres between 1/10 and1/40 and occasional titres of 1/80. Coughing was recorded in 29 animals but was probably due to severe dry conditions.

A meeting was held on 7 November, 2005 with all farmers involved in the trial. All were asked their opinion on the success of the trial. Response was nearly all favourable with no deaths reported by the farmers present. Coughing was seen in a number of herds but was believed to be caused by dust due to the unusually dry weather prevailing at the time.

Nine-month examination

All cattle were rebled. No animals had died of CBPP during the previous 3 months and coughing was rare (Tables 2.1 and 2.2). There were only 6 new seroconversions amongst 3 herds mostly in the range 1/10–1/20 though one previously seronegative cow had developed a titre of 1/80. Herd 9 from which this cow belonged contained 10 seropositive animals including one animal with a titre of 1/160 suggesting a positive active infection. In total, 60 animals were seropositive with mean titres of 1/10–1/20.

TABLE 2.1
Data on 13 treated herds over nine months

Herd	Size*	Months			
		0 No. sero+/ deaths**	3 No. sero+/ deaths	6 No. sero+/ deaths	9 No. sero+/ deaths
1	18	4/17	7/0	5/0	3/0
2	98	10/9	30/0	17/0	7/0
3	12	1/13	3/0	4/0	0/0
4	22	1/4	8/0	2/0	0/0
5	18	2/7	7/0	1/0	0/0
6	20	1/0	5/0	1/0	0/0
7	104	12/2	33/1	19/0	10/0
8	23	1/0	11/0	5/0	2/0[a]
9	63	7/4	25/1	6/0	10/0[a]
10	61	12/7	18/0	19/0	8/0
11	101	11/9	31/1	30/1	13/0[a]
12	42	2/0	18/0	17/0	5/0
13	81	2/0	14/0	16/0	2/0
Total	663	64/72	210/3	142/1	60/0

* size at treatment
** since vaccination in August 2004
[a] herds containing new seropositive cattle: Herd 8 with 1 new seropositive; Herd 9 with 4 new seropositives including 1/80; and Herd 11 with 1 new seropositive

TABLE 2.2
Summary of clinical data on treated herds over nine months

		Months		
	Prior to treatment*	3	6	9
Losses due to CBPP	72	3	1	0
Coughing	>50	9	29	0
Total seropositives	64	210	142	60

* since vaccination in August 2004

Post-mortem of selected cattle

Of the 15 cattle subjected to post-mortem after 6 months, only those animals with titres throughout the 6 months had lesions and these were chronic with extensive fibrosis (Fig 2.2) from which no mycoplasma could be detected by polymerase chain reaction (PCR) test (Table 2.3).

FIGURE 2.2
Lungs of treated cow which had been seropositive throughout study period

Lesions in left lung are chronic showing extensive fibrosis; no mycoplasmas were detected from the tissue

TABLE 2.3
Data on animals selected for slaughter after six months

Group	Number	CFT Titre*			Lesions at PM	MmmSC detected
		0	3 m	6 m		
1	142	10	80	40	fibrosis/pleuritis	-ve
	154	40	80	40	none	-ve
	371	40	160	40	adhesions	-ve
	385	80	80	80	fibrosis/pleuritis	-ve
	440	20	80	20	adhesions	-ve
2	1	0	10	0	none	-ve
	40	0	40	20	none	-ve
	116	0	10	0	none	-ve
	163	0	10	0	none	-ve
	175	0	40	0	none	-ve
3	35	0	80	40	none	-ve
	113	0	20	1	none	-ve
	184	0	0	10	none	-ve
	198	0	20	10	mild pleuritis	-ve
	366	0	40	40	none	-ve

* reciprocal serum titre
positive titre= or >10

DISCUSSION

CBPP is difficult to control: disease-free status can be maintained if vaccination coverage is high (>80 percent) and carried out at least annually. However, a lack of resources in many African countries means neither of these is possible. The use of vaccination to eliminate CBPP from a region experiencing outbreaks has never been demonstrated without other control measures including culling of affected cattle. The objective of this project was to attempt to eliminate CBPP by chemotherapy from a restricted region of the Caprivi in which mortality and morbidity were occurring despite vaccination.

We had previously shown that by treating all cattle with Advocin in an infected group, the spread of the causative mycoplasma to healthy contact cattle was greatly reduced (Hübschle *et al.,* 2004, in press). It was clear from the outset that it was not possible to include controls to determine the success of the treatment because of the following reasons:

- Untreated infected animals would pose a serious risk to the treated animals once the antibiotic effect had waned.
- Farmers would not agree to using their herds as untreated controls.
- It would not have been possible to guarantee that only authorized movements would have taken place.

Instead, it was decided to compare infection and mortality rates during the trial with that obtained in the previous six months following vaccination the previous year. The ultimate success of the project would be measured by the reduction or elimination of CBPP from this region, a task for which there was no published precedent.

The results clearly show a large reduction in clinical cases and death over the previous six-month period which is unlikely to have occurred without chemotherapeutic intervention; more encouragingly, animals at post-mortem examination, with varying but generally low serological titres, were also devoid of active lesions from which *Mmm*SC could not be detected: either lesions consisted of pleuritis and extensive fibrosis or, infrequently, small encapsulated sequestra, all indicators of a chronic state of disease which probably poses little risk to healthy contact cattle. It was postulated that chemotherapy accelerates the development of the chronic lesion but that these lesions could potentially be a source of infection (Provost *et al.,* 1987); however, current opinion holds that these lesions pose little risk: that is there is insufficient mycoplasma present to constitute an infective dose for other cattle (Nicholas *et al.,* 2000). The evidence from the present work supports this view in that all chronic lesions were sterile. In the experience of the authors, the chronic fibrotic lesions are unlike those seen in previous endemic disease states where sequestra predominate; we would propose that chemotherapeutic intervention was responsible for the enhanced resolution of the originally active lesions.

The significance of the antibody levels as detected by the CFT is more problematical. It was clear that there was a large rise in the number of seropositive animals three months following treatment. Generally speaking, there is little correlation between humoral response and protection in CBPP and death often follows once titres have exceeded 1/1280 (Nicholas *et al.,* 2004). However, in the present study antibody titres remained relatively low and very few deaths occurred, suggesting that the response may be associated with the development of protective immunity. This was probably due to a reduction of *Mmm*SC in the lungs by danafloxacin to levels of infection manageable by the host. At the end of the trial, the

number of seropositive animals decreased which is similar to that seen following vaccination where titres are often low and transient (Nicholas *et al.,* 2000).

A major problem with this type of uncontrolled study is that it is not possible to predict how the disease would have progressed without chemotherapeutic intervention. An intermediary trial planned for Zambia which would have enabled a controlled study on an infected farm did not materialize. This would have provided an opportunity to confirm our earlier findings that chemotherapy can reduce the spread of CBPP and on a larger scale. In the present study it was clear that a great deal of mortality had taken place prior to vaccination and chemotherapy, thus significantly reducing the mycoplasma load in the environment. However, mortality did continue even after vaccination.

The situation in the east of the region which was affected by a second unrelated outbreak over a year later (December 2004) after the first enables a comparison to be made, albeit qualitatively. Here the disease continues to rumble on despite vaccination and occasional and sporadic chemotherapy. However, detailed information is hard to obtain because this region is subject to seasonal flooding due to its proximity to the Zambezi.

The situation in the Caprivi is similar to that seen in Tanzania in 1990 where a single outbreak probably led to massive losses and endemic disease which persist today. CBPP was introduced by two infected heifers from southern Kenya (Mlengeya 1995). The disease was recognized quickly and animals were quarantined and surrounding cattle herds vaccinated to an unprecedented level of 90 percent thanks to help from FAO and the Netherlands. But despite this and possibly as a result of ineffective and partial use of oxytetracyclines the disease spread south and, by late 1994, 14 000 cattle had died and the disease had spread as far south as Morogoro district and Rukwa close to Zambia. It is possible that there may have also been unknown introductions of CBPP from Kenya at various times during this period but extensive vaccination appeared to have had little effect on the spread of the disease. In 2003, it was estimated that 350 000 cattle had been lost at a cost of $40 million as a result of CBPP which became the top-priority disease in Tanzania, ahead of foot-and-mouth disease (Musisi *et al.,* 2004). Zambia too was free of CBPP until 1995 when illegally introduced affected cattle from Angola and possibly from Tanzania infected Zambian herds. Like Tanzania, CBPP is now the number one priority livestock disease in Zambia.

In conclusion, it is the view of the authors that Advocin had a significant effect on the outcome of the disease in the restricted area of the Caprivi. A second new outbreak which began in the east of the Caprivi at the end of 2004 and has not been so comprehensively treated appears to suffering significant losses. However, further monitoring is required in the Linyanti region as nine months is a relatively short time and disease can erupt after a long period of clinical silence. Rapid diagnosis is, of course, crucial to any control plan in future outbreaks but once identified all cattle, should be confined and treated chemotherapeutically; movement restriction should be imposed and all at risk cattle in the district be ring vaccinated. Additionally, seriously affected cattle should be culled.

In conclusion, it appears that the host's own immune response may be responsible for most or part of the pathology seen in CBPP. Preliminary evidence that oxytetracycline was effective at reducing mycoplasma titres in lungs suggests that combined treatment of this antibiotic with Advocin should be examined ideally under controlled conditions. While it is known that OTC is mycoplasmastatic, its anti-inflammatory action combined

with Advocin's mycoplasmacidal effect may prove a highly effective combination for this complex disease.

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Presentation 3

Mathematical modelling of CBPP transmission, control methods and the potential impact of antibiotics

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INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an important disease in many of the principal pastoral areas of Africa. The clinical course of the disease and its epidemiological patterns are well known to pastoral communities (Mariner *et al.,* 2003). This paper summarizes the results of the application of participatory disease modelling to contagious bovine pleuropneumonia transmission and control.

The disease-transmission model uses a stochastic, state-transition approach that incorporated parameter estimates derived from both the scientific literature and traditional knowledge systems of pastoral communities. The models are spatially heterogeneous and incorporate three interlinked subpopulations that may represent pastoral herds or local community holdings. Stochastic models permit study of the critical community size required for the maintenance of infection.

The model was used to explore the probable impacts of various control alternatives. Particular attention was paid to the impact of movement control, vaccination and treatment. Each of these options was considered alone and in combination. Special attention was given to recent data on the effects of chemotherapy on the clinical course and transmission of CBPP. The analysis revealed that treatment of affected animals combined with vaccination of populations at risk was the approach most likely to achieve appropriate levels of control. Policy reforms are needed to enhance livestock owner access to vaccination, either private or public, and to endorse effective treatment regimens as part of integrated control strategies that include epidemiologically targeted vaccination.

PARAMETER ESTIMATION AND PARTICIPATORY EPIDEMIOLOGY

The key transmission parameter, the basic reproductive number (R_0) was estimated from the average age of seroconversion using the formula:

$$R_0 = \left(L/A\right) + 1$$

Data from two published studies (McDermott *et al.,* 1987; Zessin *et al.,* 1985) and a serosurvey conducted in south Sudan as part of this study (Mariner *et al.,* 2003) was utilized for the calculation of R_0.

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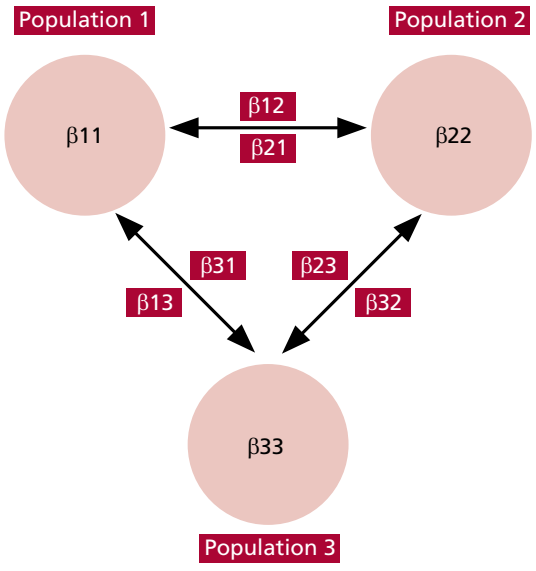
Livestock owner information from participatory epidemiological studies (Mariner and Paskin, 2000; Mariner *et al.*, 2003) and an evidence-based literature review were used to estimate other parameters (Mariner *et al.*, 2006a; Mariner *et al.*, 2006b). In the case of the evidence-based literature review, the actual data presented in papers were used as the basis for estimation of parameters.

SPATIALLY HETEROGENEOUS MODEL FOR CBPP TRANSMISSION

The models used in this analysis have six compartments and have been fully described in the literature (Mariner *et al.,* 2006a; 2006b). The compartments are susceptible (S), exposed (E), infectious (I), recovered (R), vaccinated (V) and sequestra (Q). The vaccinated state has been differentiated from the recovered state as vaccinal immunity is short-lived. The three-population structure of the model is well adapted to modelling the interaction of three pastoral herds of cattle camps. The contact structure of the populations incorporated in the model is presented in Figure 3.1. Please note that the within-subpopulation effective contact rates (β_{11}, β_{22} and β_{33}) are set based on the estimates of R_0 and were equal for all three subpopulations. The between-subpopulation effective contact rates (β_{12}, β_{13}, β_{21}, β_{23}, β_{31} and β_{32}) are all equal and set as a ratio (η) of the within-subpopulation contact rate. Unless otherwise noted, the between-subpopulation contact rate was set to 10 percent of the within-population contact rate.

The models were developed in @Risk software. All model parameter estimates are incorporated as distributions. In each iteration of a simulation, @Risk samples the input distributions

FIGURE 3.1
Population contact structure of the spatially heterogeneous CBBP model



Note: There were three interlinked subpopulations with both within- and between-subpopulation effective contact rates (β_{ij}). The between-subpopulation effective contact rates were set as a percentage (η) of the within-population contact rate.

thus providing a unique combination of input parameters for each iteration. In this way, both biological variability and the uncertainty of information were incorporated in the analysis.

A simplified formula for R_0 relates the physical contact rate (c), the probability that a contact is infectious (p) and the duration of infection (d):

$$R_0 = c*p*d$$

The duration of infection (d) is equivalent to the inverse of the recovery rate ($1/\alpha$). As $c*p = \beta$, where β is the effective contact rate, the formula can be rewritten as:

$$R_0 = \beta*d$$

Treatment programmes can have two important effects. They can either "cure" animals or they can inhibit shedding of infectious material by affected animals. Cure can be modelled as a reduction in the infectious period (d or $1/\alpha$) and an inhibition of shedding can be modelled as a reduction in p.

MODEL EXPERIMENTATION

Five types of control interventions were examined in model experimentation:

1 The impact of movement control was examined by determining the effect of different levels of between-herd contacts on the persistence of infection as a function of herd size (critical community size).
2 The impact of mass vaccination scenarios where vaccination was applied uniformly to all herds in the model.
3 The impact of elective vaccination where vaccination was applied to only one reference herd and the two in-contact herds received no intervention.
4 The potential impact of antibiotic treatment modelled as either a reduction in infectious period (d or $1/\alpha$) or a reduction in the probability that a physical contact resulted in transmission (p).
5 The impact of a combined treatment and vaccination programme.

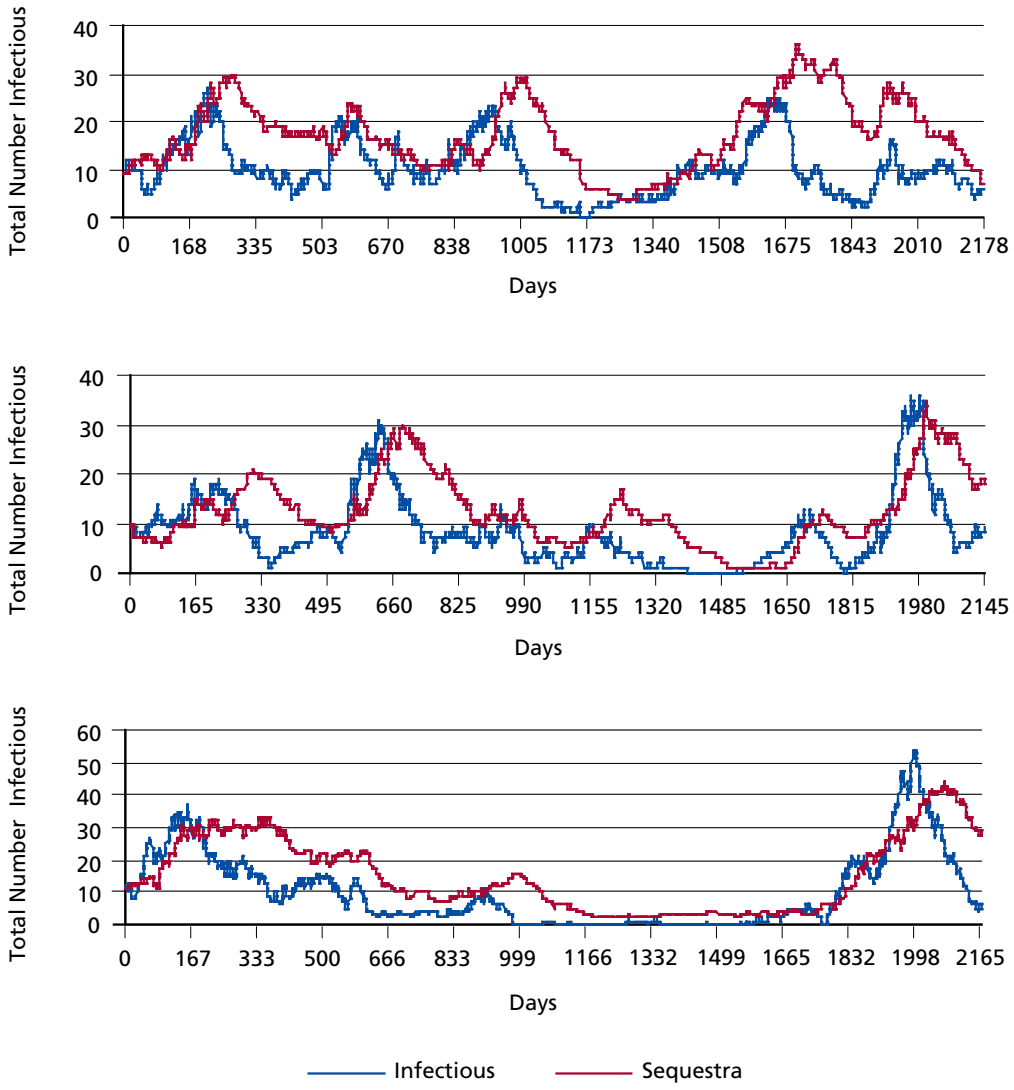
Unless otherwise stated, a herd size of 500 head and a between-herd contact rate of 10 percent of the within-herd contact rate were used. The duration of all simulations was six years.

The results of the serological analysis indicated that the value of R_0 was between 3.2 and 4.8. This range of values was used in the @Risk simulations unless otherwise noted. The full set of parameter estimates for the models have been reported previously (Mariner *et al.*, 2006a; Mariner *et al.*, 2006b).

Figure 3.2 presents representative epidemic curves for each of the three interlinked subpopulations from the same iteration of a baseline model simulation. From the curves, it is evident that peaks in the prevalence of sequestra following the peaks in the infectious case curve. As the disease fade outs in one population, it is reintroduced from one of the in-contact populations. The disease persists in the total population over time by circulating between subpopulations.

Table 3.1 presents an initial study of the impact of between-group contact on the critical community size. Final herd level prevalence, the percentage of herds that remained infected at the termination of the six-year simulation, was used as an indicator of persist-

FIGURE 3.2
Representative epidemic curves for the three subpopulations in the model (Total Number Infectious and Sequestra)



Note: The disease faded out from Subpopulations 2 and 3 but was reintroduced through contact between groups.

ence of infection in the population. When the between-subpopulation contact was set to 10 percent of the within-subpopulation contact rate, the disease could persist for the six-year duration of the simulation in herds of 100 head or less. Where between-herd contact was set to zero, herd sizes of 300 to 400 head were required to support indefinite transmission.

In Table 3.1 the "Final Herd Prevalence" was the percentage of herds where CBPP was still present at the completion of the six-year simulation. "Mean days infect" was the mean number of days that herds remained infected. "NA" indicates that it was not appropriate to calculate mean days infected as a large percentage of herds were still infected at the termination of the simulation. "ND" indicates not done. Note that in a herd size of 500

head, CBPP persisted in 75.4 percent of the herds when the between-herd contact rate was set to 10 percent of the within-herd contact rate. When the between-herd contract rate was set to zero, the disease only persisted in 10.6 percent of the herds.

Table 3.2 presents a sensitivity analysis that relates the impact of herd contact rate and herd size on the final herd prevalence of infection. Decreasing the ratio of between-herd contact to within-herd contact (η) caused reductions in the final herd prevalence of infection at any given herd size. In the case of herds of 500 head, disease persisted in 75.4 percent and 16.4 percent of herds when η was 0.1 and 0.001, respectively. This indicates that the isolation of herds must be near absolute to eliminate the effects of between-herd contact on the persistence of CBPP in populations. In relation to quarantine and movement control, this suggests that only very severe restrictions will be effective in controlling disease. This level of movement control is probably not achievable under modern socio-economic conditions in most of the affected areas of Africa. Sixty-eight percent of herds remained infected when five-year annual vaccination programmes that assumed a

TABLE 3.1
Critical community size with and without between-herd contact

Herd size	10% Inter-herd contact		No inter-herd contact	
	Final herd prevalence	Mean days infect	Final herd prevalence	Mean days infect
500	75.4	NA	10.6	NA
400	59.0	NA	5.4	NA
300	38.6	NA	1.0	1011
200	23.0	NA	0.2	812
100	5.2	1064	0	557
75	3.0	911	0	452
50	1.8	727	ND	ND
30	0	476	ND	ND

TABLE 3.2
The final herd prevalence of CBPP as a function of herd size and the ratio of between-herd contact to within-herd contact

Herd size	Ratio (η) of βbet to βwin					
	0	0.001	0.01	0.05	0.1	0.2
500	10.6	16.4	46.0	65.6	75.4	84.4
400	5.4	7.6	32.2	51.0	59.0	72.8
300	1.0	3.0	14.0	35.6	38.6	52.4
200	0.2	0.2	5.2	12.6	23.0	30.8
100	0	0.2	1.2	2.8	5.2	8.0
75	0	0	0.6	1.2	3.0	5.0
50	ND	0	0.2	0.2	0.4	0.6

vaccination efficacy of 50–80 percent were simulated. Thus, annual vaccination had almost no impact on herd level prevalence. Annual vaccination did reduce total mortality from 178 to 130 head over a six-year period. Table 3.3 presents a comparison of the efficacy of three vaccination scenarios where generous estimates of vaccine efficacy were utilized. Note that even the most aggressive biannual vaccination scenarios only halved the herd level prevalence over five years. Thus, vaccination alone is unlikely to achieve eradication, but it can result in large reductions in animal losses with important economic benefits to individual livestock owners.

Table 3.2 presents a sensitivity analysis of the effects of between-herd contact on disease persistence. "Final herd prevalence" was the percentage of herds where CBPP was still present at the completion of the six-year simulation. "ND" indicates not done. Note that at higher between-herd contact rates (5 to 20 percent), CBPP persisted in herds of 100 head or less. At higher herd sizes (300 or more head), the disease persisted despite severe between-herd contact restriction.

Table 3.3 presents the final herd prevalence of CBPP and the average total mortality experienced by a 500-head herd over a six-year simulation. Vaccination scenarios described are no vaccination, a five-year annual programme with a vaccine efficacy of 60 to 80 percent, a five-year annual vaccination programme with a vaccine efficacy of 70 to 80 percent, and a five-year biannual programme with a vaccine of 70 to 80 percent efficacy. Eighty percent of the population were vaccinated in each campaign. A vaccination efficiency (percent of vaccinations correctly applied) of 80 percent was utilized. Note that none of the programmes eradicated CBPP.

The impact of elective programmes of vaccination was also studied by applying vaccination in one reference herd and allowing disease to progress unimpeded in the two in-contact herds. The final herd prevalence of CBPP and the average total mortality experienced by a 500-head herd over a six-year simulation were used as indicators of impact. A vaccine efficacy of 50 to 80 percent was used in all these scenarios. Elective vaccination scenarios tested were no vaccination, a five-year annual programme, a two-year biannual vaccination programme, and a four-year biannual programme. Ninety percent of the population were vaccinated in each campaign. A vaccination efficiency (percent of vaccinations correctly

TABLE 3.3
Impact of vaccination in 500 head herds where between-herd contact was 10 percent of within-herd contact

Scenario	Final herd prevalence	Average mortality
No vaccination	75.4	178
Annual–5 year 60–80% efficacy	68.2	127
Annual–5 year 70–80% efficacy	28.0	74
Biannual–5 year 70–80% efficacy	21.2	66

applied) of 90 percent was utilized. None of the programmes had major effects on the final herd prevalence of disease as the vaccinated herd was continually re-infected by the non-vaccinated herds. The elective vaccination programme did greatly reduce mortality within the vaccinated herd. Total mortality was reduced from 178 to 124 heads when annual elective vacation was practised and to 109 heads when biannual elective vaccination was undertaken. Although elective vaccination was unlikely to contribute to disease eradication, it was of major benefit to livestock owners.

When the impact of treatment was modelled as a 50 percent reduction in the infectious period ($1/\alpha$), the final herd prevalence of CBPP was reduced to 33.2 percent, the number of animals with sequestra was reduced from 293 to 185 and the total mortality was reduced from 178 to 64. This impact on mortality was greater than any vaccination regimen and the reduction in herd level prevalence was comparable to aggressive biannual vaccination programmes. Note that as treatment reduces the total number of cases, the number of animals with sequestra in the population also declined. This outcome is logical but runs counter to the common misconception that treatment favours the creation of chronically infected animals. The mass effects of treatment actually decrease the prevalence of animals with sequestra.

Simulations that combined vaccination and a 50 percent reduction of the duration of the infectious period came close to eradicating CBPP. At the end of the six-year simulation that incorporated four years of biannual vaccination, 0.4 percent of herds were infected. This suggests that programmes that combine treatment of infected cases with vaccination of the general population would be very effective.

In vitro antibiotic trials have shown that CBPP is susceptible to a wide range of antibiotics (Ayling *et al.*, 2000). *In vivo* antibiotic trials (Hübschle *et al.*, 2004; Hübschle *et al.,* 2006) and field pilot programmes indicate that treatment can have a dramatic effect on transmission and result in a major reduction in the effective reproductive number to values well below 1. Based on this new information, it was decided to examine the effect of treatment modelled as a reduction in 'p' the probability that physical contacts result in transmission. Table 3.4 presents the results of simulations where treatment effect was modelled as a reduction in the probability that physical contacts result in transmission. As the

TABLE 3.4
Effect of reducing probability that a physical contact resulted in transmission (p) on R nought, the final herd prevalence, total number of cases, rate that recovered cases form sequestra, total number of sequestra and total mortality in a six-year simulation

p	R0	Final herd prevalence	Total cases	Sequest rate	Total sequest	Total mortality
p	4.10	75.4	602	0.49	293	178
p/2	2.1	61.8	342	0.48	164	114
p/4	1.0	0	41	0.40	16	17

Note: As p was reduced by 50 percent and 75 percent, the average value of R0 was 2.1 and 1.0, respectively. Reducing p by 50 percent, resulted in important reductions in the total number of cases and the total mortality, but with a final herd prevalence of 61.8 percent did not contribute greatly to eradication. Reducing p by 75 percent eradicated the disease.

analysis shows, such important effects on transmission (reductions in the effective contact rate below 1) are consistent with eradication of the disease.

Formation of sequestra is part of the normal healing process in the recovery from CBPP. When the effect of antibiotics was modelled as a reduction of p, there were fewer cases of CBPP and the subsequent number of sequestra present in the population was reduced. This effect was even greater than in the cases where treatment was modelled as a decrease in the duration of the infectious disease. This was because a decrease in the duration of the infectious period is equivalent to an increased recovery rate.

CONCLUSIONS AND RECOMMENDATIONS

It is important to have accurate and realistic expectations of the probable impact of CBPP control options. The research suggest that the impact of mass vaccination as an eradication tool is limited in the current political and social climate of Africa, but that vaccination has important benefits for individuals. Based upon the results of the field investigations, modelling work and literature review, the following recommendations can be made relative to CBPP control:

- Mass vaccination programmes based on the T1/44 vaccine that are not combined with other interventions are unlikely to eradicate CBPP even when aggressively applied biannually over a five-year period.
- A regular programme of mass vaccination or elective vaccination will have a major impact on morbidity and mortality losses and is of economic benefit to livestock owners.
- Proven treatment regimes (Hübschle *et al.*, 2006) have considerable impact on the transmission of CBPP and reduce the effective reproductive numbers of the disease.
- Treatment programmes can reduce both the prevalence of CBPP and the morbidity, mortality and production losses associated with CBPP infection.
- The data on treatment indicate that that treatment may have more value as a CBPP control tool than current vaccines, However, larger-scale pilot programmes are required to substantiate the indications of the trials.
- Combined vaccination and treatment programmes offer greater impact (up to 44 per cent over annual vaccination campaigns alone) than either approach alone.
- Regular programmes of elective control lead to reduced disease morbidity, mortality and production losses even if in-contact herds do not practise vaccination.
- As open-ended annual mass vaccination programmes are unsustainable and ineffective as practised, vaccination strategies should be targeted to achieve specific epidemiological goals within an achievable national plan.
- Levels of movement control attainable under current socio-economic conditions in sub-Saharan Africa will not have a major impact on CBPP prevalence and policy-makers are encouraged to focus on more realistic and achievable control options.

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Presentation 4

Comparative studies on the *in vitro* antimicrobial sensitivities of *Mycoplasma mycoides* subsp. *mycoides* SC type and *Mycoplasma bovis*

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INTRODUCTION

A major recommendation of the FAO-OIE-AU/IBAR-IAEA Consultative Group on contagious bovine pleuropneumonia (CBPP) at the 3rd Meeting in Rome in November 2003 was the need to carry out minimum inhibition concentration (MIC) and minimum mycoplasmacidal concentration (MMC) studies on current African strains of *Mycoplasma mycoides* subsp. *mycoides* small colony type (*Mmm*SC). The aim was to facilitate the selection of candidate chemotherapeutic agents for possible future use in the control of contagious bovine pleuropneumonia (FAO, 2004). Previously it has been reported that antimicrobial treatment may alleviate the clinical signs, but not prevent the spread of infection, thus favouring the creation of chronic carriers (Provost *et al.*, 1987). Yaya *et al.* (2004) also demonstrated that tetracycline does not eliminate CBPP. Hence, in several African countries treatment of CBPP-affected cattle with antimicrobials was officially discouraged although it is known to be widely practised (Mariner and Catley, 2004). However, subsequently a number of newer antimicrobials, such as the fluoroquinolones, have been developed which may be more effective in controlling CBPP than previous products. New work on the role of chemotherapy is especially urgent as the development of new CBPP vaccines is still a long way off.

Previous MIC studies on *Mmm*SC have been limited in the number of isolates and antimicrobials tested (Ayling *et al.,* 2000a; Mazzolini *et al.,* 1997). In the present study MIC and MMC values were obtained for 41 *Mmm*SC African and European strains isolated over the last 10 years against 21 antimicrobials. This data will be useful for selecting potentially effective antimicrobials against CBPP and also provide a baseline of MIC values to monitor and assess development of antimicrobial resistance. Several reports of antimicrobial resistance by *Mycoplasma species* have been made (Ayling *et al.*, 2000b; Francoz *et al.*, 2005; Suzuki *et*

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al., 2006). However, to date none has been reported for *Mmm*SC recovered from the field. A comparison of MIC values for *Mmm*SC and 20 recent UK *M. bovis* isolates are compared.

METHODS

Details of the 41 isolates of *Mmm*SC used in this study are given in Table 4.1. Twenty-seven of these were isolated in Africa between 1996 and 2004. Twenty *M. bovis* strains isolated in the UK in 2005 were also tested. The field isolates had been minimally passaged in Eaton's broth medium (Nicholas and Baker, 1998) and stored at –70°C until required. Species identification was confirmed by standard mycoplasma identification techniques (Bashiruddin *et al.,* 1994; Nicholas *et al.,* 1996). MICs and MMCs were determined as described previously using a microbroth dilution method (Ayling *et al.,* 2005). The antimicrobials used were the fluoroquinolones: ciprofloxacin (CIP), danofloxacin (DAN), enrofloxacin (ENRO), naladixic acid (NAL), and norfloxacin (NOR); the tetracycline: oxytetracycline (OXYTET); the aminoglycosides: amikacin (AMK), gentamycin (GEN), streptomycin (STR), and tobramycin (TOB); the macrolides: erythromycin (ERY), tilmicosin (TIL), tylosin (TYL); the chloramphenicol and derivatives: chloramphenicol (CHL), florfenicol (FLO); the lincosamides, mutalins and aminocyclitols: clindamycin (CLI), lincomycin (LIN), spectinomycin (SPT); cephalosporin, beta lactam antibiotic: cephalothin (CEF); the ansamycin: rifampin (RIF); and the benzylpyrimidine: trimethroprim combined with the sulfonamide sulfamethoxazole (SXT).

RESULTS

The individual isolate results for the *Mmm*SC MIC and MMCs are given in Table 4.1 for the most effective antimicrobials which exclude AMK, CEF, GEN, NAL, RIF, STR, TOB and SXT. These results are summarized in Table 4.2, which gives the range, 50 percent and 90 percent values for MIC and MMC. The results of the effective antimicrobials are shown graphically in Figure 4.1, which clearly show the distribution of the MIC values and the cumulative MIC and MMC values. The *M. bovis* MIC results are given within a range of 50 percent and 90 percent in Table 4.3. A comparison of selected antimicrobials between *Mmm*SC and *M. bovis* are given in Figure 4.2.

DISCUSSION

Different bacterial species and indeed different *Mycoplasma species* respond differently to antimicrobials and have different MIC values. Al Momani *et al.* (2006) demonstrated that members of the closely related *M.* "*mycoides* cluster" that cause contagious agalactia syndrome in small ruminants responded differently to antimicrobials *in vitro* even though some isolates were from the same animal.

This study provides *in vitro* data for *Mmm*SC isolates to identify potentially useful antimicrobials in the treatment of CBPP. This also provides a baseline level of antimicrobial sensitivity so that any development of antimicrobial resistance can be monitored. One of the concerns connected with using antimicrobials to either treat or reduce the spread of CBPP is the potential for development of antimicrobial resistance. These results demonstrate that the MIC values obtained for the recent African isolates, with the other *Mmm*SC isolates tested showed no major differences in the MIC range or the MIC_{50} values obtained. This includes isolates obtained from Portugal in 1997. Although Portugal has a slaughter

and compensation policy for CBPP, it is likely that the affected cattle would have had more exposure to antimicrobials than animals in Africa. It is therefore encouraging that no antimicrobial resistance had been identified in *Mmm*SC isolates.

When compared to the MIC values obtained for UK isolates of *M. bovis*, major differences between the MIC values for the two organisms are apparent, particularly with tilmicosin, erythromycin, chloramphenicol, and oxytetracycline, where all the *Mmm*SC are more sensitive than most of the *M. bovis* isolates. This may be because *M. bovis* is inherently more resistant to antimicrobials or more probably that resistance has developed due to the extensive use of antimicrobials in the UK.

M. bovis and *Mmm*SC are related, being in the class *Mollicutes* and the family *Mycoplasmataceae.* Both organisms also cause serious disease in cattle. Taxonomically they are in different clusters with *Mmm*SC in the spiroplasma cluster and *M. bovis* in the hominis cluster (Pettersson *et al*., 1996). Other differences may also have an effect on how the organisms respond to antimicrobials, or acquire antimicrobial resistance. The genome sizes are different with *Mmm*SC having a genome size of 1.14 Mbp and *M. bovis* 961+/-18.9 kb. Molecular typing methods have found few ways of differentiating *Mmm*SC isolates (Frey *et al*., 1996, Yaya *et al*., 2006) indicating the stability of the *Mmm*SC genome, whereas the variability between *M. bovis* isolates has been demonstrated by several different molecular typing methods (McAuliffe *et al.,* 2004). The presence of variable surface antigens and ability to incorporate insertion elements into the genome may also affect the two organisms' ability to respond to antimicrobials or acquire resistance.

Previous work (Lee *et al.,* 1987; Ayling, 2002) has demonstrated that antimicrobial resistance to spectinomycin can be induced in a few steps by culturing at sub MIC concentrations of spectinomycin. This would therefore make spectinomycin and related antimicrobials unsuitable for regular use. Whilst *Mmm*SC and *M. bovis* are different in many respects, it is useful to compare the MIC data for *M. bovis* as it may give an insight as to the possible generation of antimicrobial resistance by *Mmm*SC if antimicrobials were used indiscriminately. Whilst *Mmm*SC is sensitive *in vitro* to TIL, ERY and OXYTET, the insensitivity/resistance by *M. bovis* to these antimicrobials would indicate they may not be the best antimicrobials to use in controlling CBPP. Therefore, the newer antimicrobials, such as the fluoroquinolones DAN and ENRO may be worthy candidates for *in vivo* trials. Whilst UK *M. bovis* isolates have not yet developed resistance to DAN and ENRO, some higher MIC values have been detected to the related fluoroquinolones NOR and CIP. Le Carrou *et al*. (2006) describe possible resistance by *M. gallisepticum* to ENRO, thus emphasizing the need for strategic planned treatment if antimicrobials are to be used in the control of CBPP.

This study therefore provides a more accurate basis for the selection of antimicrobials against *Mmm*SC/CBPP *in vivo*. Whilst much debate will continue about recommending the use of antimicrobials to treat CBPP, it has already been demonstrated *in vivo* that antimicrobials can reduce the spread of CBPP, even if they are not effective at curing the condition (Hübschle *et al*., 2004, 2006a). However, more recent work in Namibia has shown that the use of DAN on all animals in an affected herd may eventually lead to a significant reduction in clinical cases in fresh outbreaks combined with vaccination (Hübschle *et al*., 2006b). Therefore, strategic antimicrobial treatment combined with other measures such as vaccination, movement control and culling may help in the elimination of CBPP.

TABLE 4.1
Individual MIC and MMC values (in brackets) for *Mycoplasma mycoides* subsp. *mycoides* SC isolates against 13 antimicrobials. Results in µg/ml.

		ANTIMICROBIAL												
STRAIN REF.	SOURCE	TIL	OXYTET	ERY	ENRO	CLI	DAN	CIP	LIN	CHL	FLO	NOR	SPT	TYL
V5	Australia, 1936	<0.06 (0.5)	0.12 (16)	0.25 (>32)	0.12 (4)	0.25 (8)	0.25 (4)	0.25 (8)	1 (32)	2 (32)	1 (16)	2 (32)	2 (64)	64
KH3J	Sudan, 1940	<0.06 (0.5)	0.12 (16)	<0.12 (32)	0.25 (4)	0.25 (8)	0.25 (16)	0.5 (8)	0.5 (32)	2 (32)	2 (16)	8 (>32)	16 (>64)	>64
Afade	Chad, 1968	<0.06 (0.25)	0.12 (8)	<0.12 (>32)	0.25 (4)	0.25 (32)	0.25 (8)	0.25 (8)	1 (32)	2 (32)	1 (16)	2 (>32)	8 (>64)	16
2091	France, 1984	<0.06 (8)	<0.06 (4)	<0.12 (>32)	0.25 (8)	0.25 (32)	0.25 (8)	0.25 (8)	0.5 (>32)	1 (32)	1 (16)	2 (32)	16 (>64)	4
2022	France, 1984	<0.06 (0.5)	0.12 (8)	<0.12 (>32)	0.25 (4)	0.25 (8)	0.25 (8)	0.5 (8)	2 (32)	2 (32)	1 (16)	2 (32)	16 (>64)	64
138/5	Italy, 1992	<0.06 (2)	<0.06 (4)	<0.12 (>32)	0.25 (4)	0.25 (8)	0.25 (4)	0.25 (8)	2 (32)	2 (32)	1 (16)	2 (32)	16 (>64)	8
197	Italy, 1992	<0.06 (0.5)	<0.06 (8)	<0.12 (8)	0.12 (4)	<0.12 (8)	0.12 (8)	<0.12 (8)	<0.12 (32)	0.25 (32)	0.25 (16)	8 (>32)	4 (>64)	64
Madrid	Spain, 1993	<0.06 (0.25)	<0.06 (16)	0.25 (>32)	0.12 (4)	0.25 (8)	0.12 (8)	0.25 (8)	1 (32)	1 (32)	0.5 (16)	1 (32)	4 (>64)	16
B103	Portugal, 1986	<0.06 (0.5)	0.12 (4)	<0.12 (8)	0.12 (4)	<0.12 (32)	0.25 (8)	0.25 (8)	0.5 (32)	0.5 (32)	0.5 (16)	2 (>32)	8 (>64)	16
M545/91	Portugal, 1991	<0.06 (0.25)	0.12 (8)	<0.12 (8)	0.25 (4)	0.25 (8)	0.25 (8)	<0.12 (8)	0.5 (32)	1 (32)	1 (32)	1 (32)	4 (>64)	>64
B526	Portugal, 1993	<0.06 (1)	<0.06 (16)	<0.12 (>32)	0.25 (4)	<0.12 (32)	0.25 (16)	0.25 (8)	1 (>32)	1 (32)	2 (16)	2 (>32)	16 (>64)	16
6305	Portugal, 1993	<0.06 (0.5)	<0.06 (8)	<0.12 (8)	0.25 (8)	<0.12 (8)	0.25 (8)	0.25 (8)	0.25 (32)	1 (32)	0.5 (16)	2 (>32)	16 (>64)	16
410	Portugal, 1997	<0.06 (0.5)	<0.06 (4)	<0.12 (32)	0.25 (4)	0.25 (32)	0.25 (4)	0.25 (8)	0.5 (>32)	2 (32)	2 (16)	2 (>32)	16 (>64)	64
427	Portugal, 1997	<0.06 (0.25)	0.12 (16)	0.25 (8)	0.12 (8)	0.25 (8)	0.25 (8)	0.25 (8)	2 (>32)	2 (>32)	1 (32)	1 (>32)	4 (>64)	2
M375	Botswana, 1996	<0.06 (0.25)	0.12 (8)	0.25 (2)	0.12 (4)	0.25 (8)	0.12 (8)	0.25 (8)	0.5 (8)	0.25 (32)	0.5 (16)	2 (>32)	4 (>64)	8
N6	Botswana, 1996	<0.06 (0.25)	<0.06 (8)	<0.12 (2)	0.25 (4)	0.5 (2)	0.25 (16)	0.25 (8)	0.5 (8)	2 (32)	1 (16)	2 (32)	4 (>64)	0.25
TAN 8	Tanzania, 1996	<0.06 (0.25)	<0.06 (8)	<0.12 (1)	0.12 (2)	<0.12 (8)	0.25 (4)	0.25 (32)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	2
1S6	Tanzania, 1997	<0.06 (0.25)	<0.06 (4)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (8)	0.25 (8)	0.5 (8)	1 (32)	1 (16)	1 (32)	4 (>64)	16
1S18	Tanzania, 1997	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	0.5 (32)	1 (16)	2 (32)	8 (>64)	8
1S5	Tanzania, 1998	0.12 (0.5)	0.06 (64)	<0.12 (8)	0.25 (2)	0.5 (1)	0.25 (32)	<0.12 (8)	0.25 (>32)	1 (32)	1 (16)	1 (32)	4 (>64)	0.5
1S26	Tanzania, 1998	<0.06 (0.25)	0.12 (32)	<0.12 (8)	0.12 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	1 (32)	2 (16)	2 (32)	8 (>64)	16
1S31	Tanzania, 1998	<0.06 (8)	0.12 (32)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.12 (4)	<0.12 (8)	<0.12 (8)	1 (32)	1 (16)	2 (32)	4 (>64)	16

TABLE 4.1 (CONTINUED)

Individual MIC and MMC values (in brackets) for *Mycoplasma mycoides* subsp. *mycoides* SC isolates against 13 antimicrobials. Results in µg/ml.

		ANTIMICROBIAL												
STRAIN REF.	SOURCE	TIL	OXYTET	ERY	ENRO	CLI	DAN	CIP	LIN	CHL	FLO	NOR	SPT	TYL
1S33	Tanzania, 1998	<0.06 (8)	<0.06 (8)	<0.12 (2)	0.12 (4)	<0.12 (2)	0.12 (4)	0.25 (32)	0.5 (8)	1 (>32)	0.5 (8)	1 (32)	8 (>64)	1
1S21	Tanzania, 1999	0.12 (8)	0.5 (32)	<0.12 (1)	0.12 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (32)	2 (32)	1 (16)	2 (32)	8 (>64)	64
1S22	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (32)	1 (32)	1 (16)	2 (32)	8 (>64)	64
1S23	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (2)	0.5 (2)	<0.12 (8)	0.5 (32)	0.25 (8)	0.25 (32)	1 (32)	8 (>64)	2 (32)	16 (>64)	2
1S24	Tanzania, 1999	<0.06 (0.25)	0.12 (16)	<0.12 (2)	0.12 (4)	<0.12 (2)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (32)	1 (32)	2 (32)	4 (>64)	4
1S25	Tanzania, 1999	<0.06 (1)	0.12 (32)	<0.12 (>32)	0.12 (8)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	2 (32)	1 (16)	2 (32)	4 (>64)	2
1S27	Tanzania, 1999	0.12 (0.25)	0.25 (32)	<0.12 (8)	0.12 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.5 (8)	2 (32)	1 (32)	2 (32)	8 (>64)	64
1S28	Tanzania, 1999	<0.06 (0.25)	0.25 (32)	0.25 (2)	0.12 (4)	0.25 (2)	0.25 (4)	0.25 (8)	1 (8)	2 (32)	2 (16)	2 (32)	8 (>64)	16
1S29	Tanzania, 1999	<0.06 (0.25)	0.12 (32)	<0.12 (2)	0.12 (2)	0.25 (2)	0.25 (4)	0.25 (8)	0.5 (32)	1 (32)	1 (16)	2 (32)	4 (>64)	16
1S30	Tanzania, 1999	<0.06 (16)	0.12 (64)	<0.12 (1)	0.12 (2)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	1 (>32)	0.5 (8)	2 (>32)	4 (>64)	16
1S32	Tanzania, 1999	<0.06 (0.25)	<0.06 (16)	<0.12 (2)	0.12 (2)	<0.12 (2)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (32)	0.5 (16)	2 (32)	4 (>64)	16
1S34	Tanzania, 1999	<0.06 (0.12)	<0.06 (32)	<0.12 (1)	0.12 (8)	<0.12 (2)	0.12 (4)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	16
1S35	Tanzania, 1999	<0.06 (0.25)	0.12 (32)	<0.12 (8)	0.12 (2)	<0.12 (2)	0.25 (16)	0.25 (8)	0.5 (8)	0.5 (32)	0.5 (16)	2 (32)	8 (>64)	16
26/3/01	Namibia, 2001	<0.06 (0.5)	<0.06 (4)	<0.12 (2)	0.25 (2)	0.5 (8)	0.25 (16)	0.5 (8)	1 (8)	2 (32)	1 (16)	2 (32)	8 (>64)	8
28/3/01	Namibia, 2001	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (2)	0.25 (8)	0.5 (16)	0.5 (8)	0.5 (8)	1 (32)	1 (16)	>32 (>32)	8 (>64)	2
SF0177	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (8)	0.12 (4)	0.25 (8)	0.25 (8)	0.5 (8)	0.5 (32)	1 (32)	0.5 (16)	8 (>32)	8 (>64)	8
9809	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (2)	<0.12 (2)	0.25 (32)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (>32)	8 (>64)	4
Okahao	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (8)	0.25 (8)	0.25 (8)	0.25 (8)	0.5 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	ND
Matapi	Namibia, 2004	<0.06 (0.25)	<0.06 (4)	<0.12 (2)	0.25 (4)	0.25 (8)	0.25 (8)	0.25 (8)	0.25 (8)	1 (32)	0.5 (16)	2 (32)	4 (>64)	0.12

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FIGURE 4.1
Graphs showing MIC distribution, cumulative MIC and MMC values for *Mmm*SC

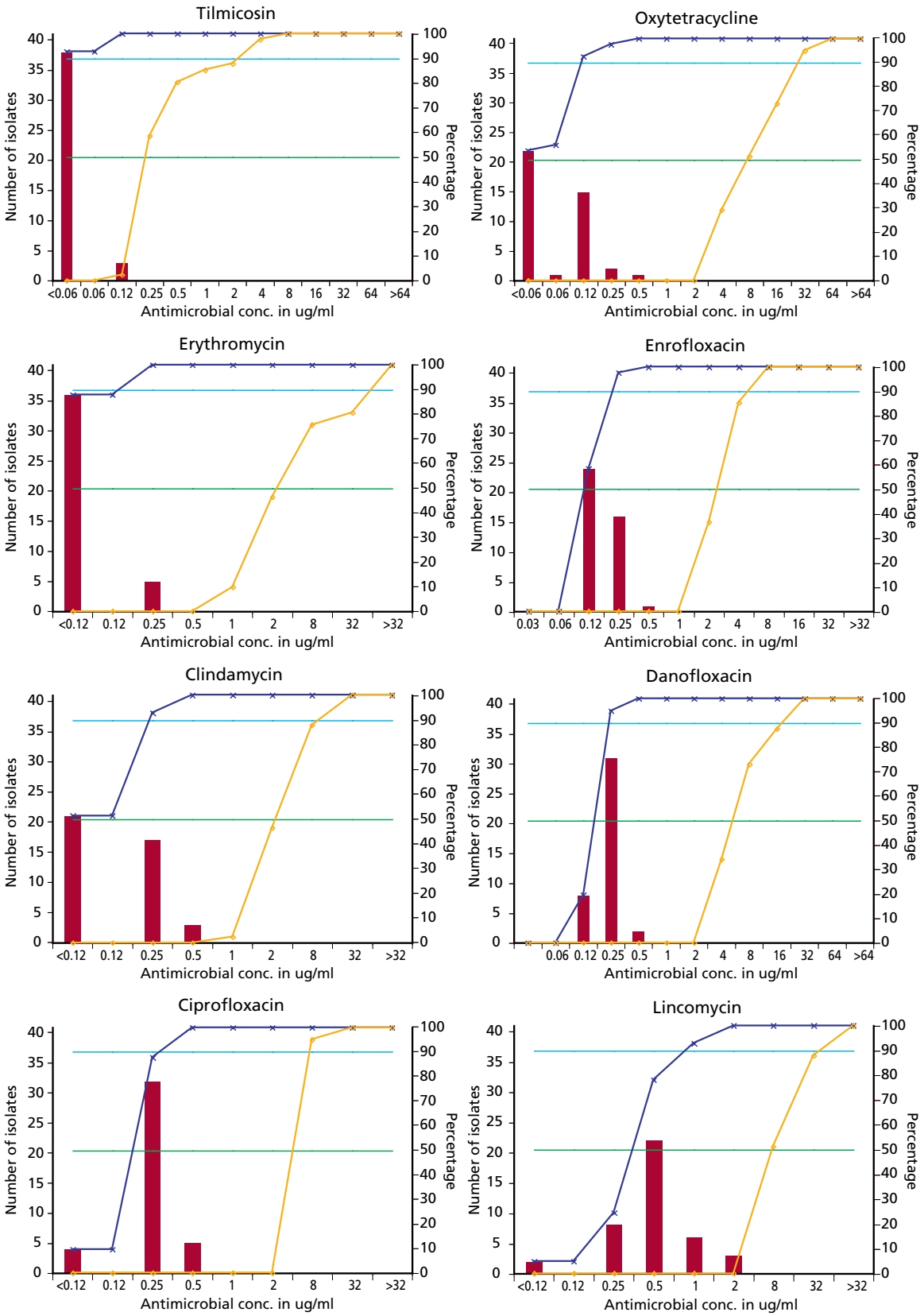

FIGURE 4.1 (CONTINUED)

Graphs showing MIC distribution, cumulative MIC and MMC values for *Mmm*SC

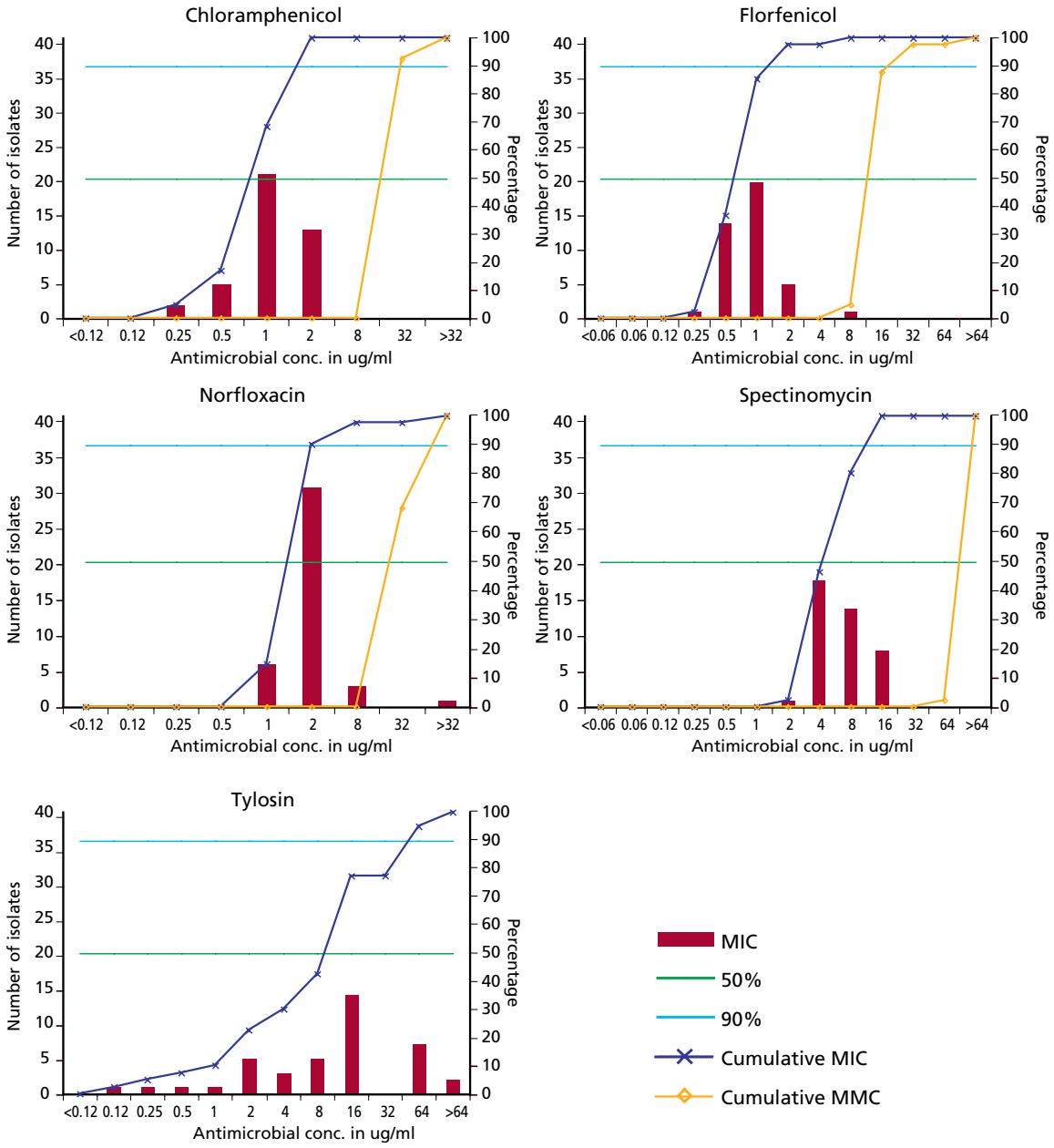

TABLE 4.2

Summary of MIC range, MIC_{50} and MIC_{90} values, MMC range and MMC_{50} and MMC_{90} values in µg/ml for 50 isolates of *Mycoplasma mycoides* subsp. *mycoides* SC

Antimicrobial	MIC Range	MIC 50	MIC 90	MMC Range	MMC 50	MMC 90
Tilmicosin (TIL)	<0.06– 0.12	<0.06	<0.06	0.12– 16.00	0.25	2.00
Oxytetracycline (OXYTET)	<0.06– 0.50	<0.06	0.12	4.00– 64.00	16.00	32.00
Erythromycin (ERY)	<0.12– 0.25	<0.12	0.25	1.00– >32.00	8.00	>32.00
Enrofloxacin (ENRO)	0.12– 0.50	0.12	0.25	2.00– 8.00	4.00	8.00
Clindamycin (CLI)	<0.12– 0.50	0.25	0.25	1.00– 32.00	8.00	32.00
Danofloxacin (DAN)	0.12– 0.50	0.25	0.25	4.00– 32.00	8.00	16.00
Ciprofloxacin (CIP)	<0.12– 0.50	0.25	0.50	8.00– 32.00	8.00	8.00
Lincomycin (LIN)	<0.12– 2.00	0.50	1.00	8.00– >32.00	8.00	>32.00
Chloramphenicol (CHL)	0.25– 2.00	1.00	2.00	32.00– >32.00	32.00	32.00
Florfenicol (FLO)	0.25– 8.00	1.00	2.00	8.00– >64.00	16.00	32.00
Norfloxacin (NOR)	1.00– >32.00	2.00	8.00	32.00– >32.00	32.00	>32.00
Spectinomycin (SPT)	2.00– 16.00	8.00	16.00	64.00– >64.00	>64.00	>64.00
Tylosin (TYL)	0.12 –>64.00	16.00	64.00	Not tested	Not tested	Not tested
Tobramycin (TOB)	8.00– >32.00	32.00	32.00	>32.00– >32.00	>32.00	>32.00
Gentamycin (GEN)	16.00– 64.00	32.00	64.00	>64.00– >64.00	>64.00	>64.00
Naladixic acid (NAL)	8.00– >32.00	>32.00	>32.00	>32.00– >32.00	>32.00	>32.00
Cephalothin (CEF)	16.00– >64.00	>64.00	>64.00	>64.00– >64.00	>64.00	>64.00
Streptomycin (STR)	32.00– >32.00	32.00	32.00	>32.00– >32.00	>32.00	>32.00
Rifampin (RIF)	32.00– >32.00	>32.00	>32.00	>32.00– >32.00	>32.00	>32.00
Amikacin (AMK)	>32.00– >32.00	>32.00	>32.00	>32.00– >32.00	>32.00	>32.00
Trimethoprim / sulfamethoxazole (SXT)	>32/608– >32/608	>32/608	>32/608	>32/608– >32/608	>32/608	>32/608

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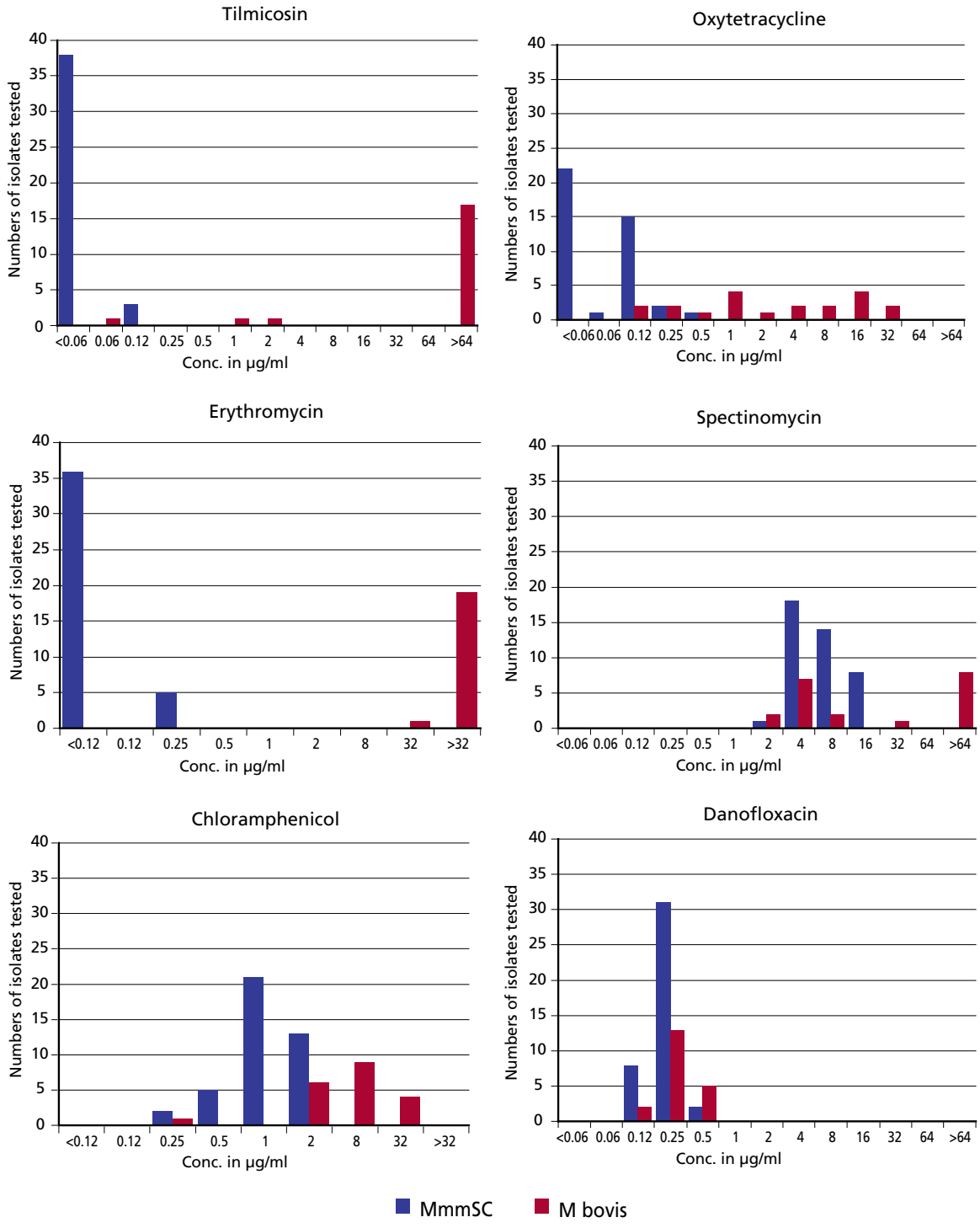
TABLE 4.3

Summary of MIC range, MIC_{50} and MIC_{90} values in µg/ml for 20 UK isolates of *Mycoplasma bovis*

Antimicrobial	MIC range	MIC 50	MIC 90
Tilmicosin (TIL)	<0.06– >64.00	>64.00	>64.00
Oxytetracycline (OXYTET)	0.12– 32.00	2.00	16.00
Erythromycin (ERY)	32.00– >32.00	>32.00	>32.00
Enrofloxacin (ENRO)	0.12– 1.00	0.25	0.50
Clindamycin (CLI)	0.25– >32.00	>32.00	>32.00
Danofloxacin (DAN)	0.12– 0.50	0.25	0.25
Ciprofloxacin (CIP)	<0.25– 2.00	1.00	1.00
Lincomycin (LIN)	1.00– >32.00	>32.00	>32.00
Chloramphenicol (CHL)	0.25– 32.00	8.00	32.00
Florfenicol (FLO)	2.00– 16.00	4.00	8.00
Norfloxacin (NOR)	1.00– 32.00	8.00	32.00
Spectinomycin (SPT)	2.00– >64.00	8.00	>64.00
Tylosin (TYL)	ND ND	ND	
Tobramycin (TOB)	>32.00 >32.00	>32.00	>32.00
Gentamycin (GEN)	0.50– 32.00	4.00	8.00
Naladixic acid (NAL)	32.00– >32.00	>32.00	>32.00
Cephalothin (CEF)	>64.00– >64.00	>64.00	>64.00
Streptomycin (STR)	32.00– >32.00	>32.00	>32.00
Rifampin (RIF)	32.00– >32.00	32.00	>32.00
Amikacin (AMK)	2.00– >32.00	32.00	>32.00
Trimethoprim / sulfamethoxazole (SXT)	>32/608– >32/608	>32/608	>32/608

FIGURE 4.2
Graphs showing comparative MIC distribution of *Mmm*SC and UK *M. bovis* isolates



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Presentation 5

Fine differentiation of *Mycoplasma mycoides* subsp. *mycoides* SC strains by multilocus sequence typing

Yaya, A.,[1] Manso-Silvan, L.,[2] Peyraud, A.[2] and Thiaucourt, F.[2]

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a disease affecting cattle and domestic buffaloes caused by a mycoplasma: *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC). This disease belongs to the OIE list of diseases that are important to international trade. CBPP is mostly found in Africa, where its distribution has expanded in the last ten years in spite of prophylactic measures that include vaccination campaigns. It has been eradicated from all other continents, although the situation in Asia still needs to be ascertained. In Europe some outbreaks were reported in the late 1990s and preliminary studies concluded that *Mmm*SC strains found in Europe were of a distinctive type. This may indicate that previous eradication campaigns were not as successful as considered at the time and that *Mmm*SC strains circulated unnoticed for many years, since no CBPP outbreaks had been recorded in Europe between 1982 and 1993. The development of new tools for typing *Mmm*SC strains, capable of tracing back the origin of the outbreaks, is much needed.

Multilocus sequence typing (MLST) is a nucleotide-sequence based approach for the unambiguous characterization of isolates of bacteria and other organisms. The aim of MLST is to find loci on the bacterial chromosome that exhibit sufficient polymorphism as to allow the description of various alleles on each locus. The combination of a number of loci, usually around seven, allows a fine typing of the pathogens under study.

METHODS

An initial MLST scheme had been designed in 2003 in our laboratory. Since then, the complete sequence of the reference strain PG1 has been published and the whole genome of a pathogenic strain of African origin is under assembly in our laboratory. The comparison between these two sequences has permitted the selection of new loci for MLST typing of *Mmm*SC strains. The insertion elements were removed, leaving a total of 800 Kb for comparison. Only 300 polymorphic positions were identified, from which 30 potential loci were chosen on housekeeping genes, on genes coding for surface proteins and on non-coding sequences. The potential of these loci was tested on a limited subset of *Mmm*SC strains of

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various origins and the selected loci were then sequenced on a representative number of strains spanning the entire distribution of CBPP. The allelic profiles were then analysed by "eBURST" to group the strains and to define possible ancestral strains.

The preliminary validation showed that half of the polymorphic sites were specific to the reference strain PG1, possibly as a consequence of multiple *in vitro* passages, so they could not be used for the MLST scheme. Eight loci were chosen, the resolving power of which varied greatly, both in terms of the number of alleles found (from two to six; Figure 5.1) and in terms of the distribution of the strains within these alleles. The 48 strains studied could be differentiated into 32 MLST profiles and three main groups (Figures 5.1 and 5.2). Interestingly, all the European *Mmm*SC strains tested exhibited a specific allele in two loci. This confirmed that they are different from the African strains tested. In addition, one strain of European origin could be differentiated from the others based on a specific polymorphic site. A strain isolated in 1967 in Europe lies at an intermediate position between the European group and a group comprising strains isolated in the south of Africa, and strains isolated in the past in Australia, India and the Horn of Africa. Another locus allowed the segregation of the Sub-Saharan strains from all the other. The geographical position of each strain and type is given in Figure 5.3.

DISCUSSION

The MLST is a very robust technique. PCR can be run with samples such as DNA or dried material that do not need a cold chain upon transport. PCR products can also be shipped without any biological hazard and sequencing results may be sent via the internet. The results obtained with a representative number of *Mmm*SC strains have shown that recent CBPP outbreaks in Europe were certainly due to resurgence of the disease and not because of a reintroduction of the agent. This calls for renewed surveillance efforts based mostly

FIGURE 5.1
Example of a locus with three alleles and allelic profiles of 48 *Mmm*SC strains

FIGURE 5.2
Example of *Mmm*SC strain grouping according to "eBURST"

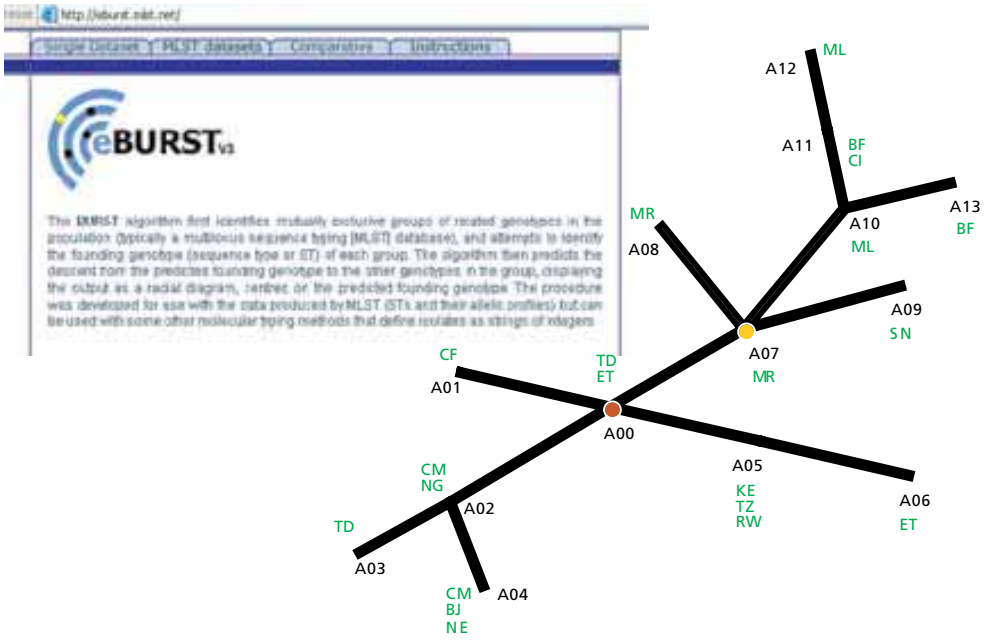
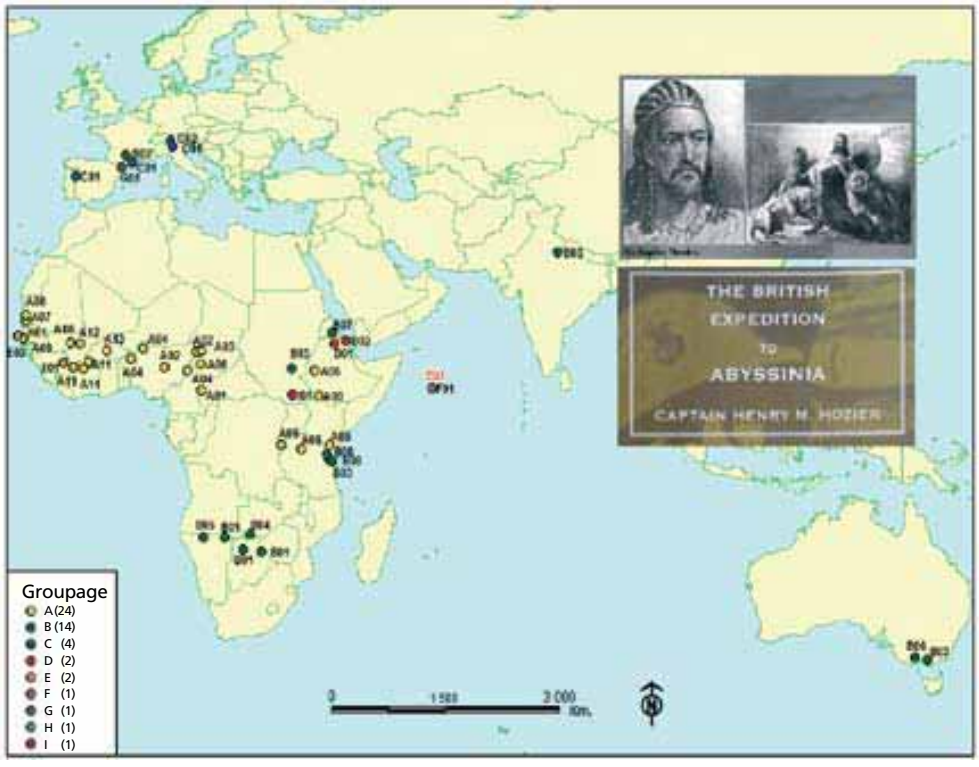


FIGURE 5.3
Geographic distribution of *Mmm*SC strains and MLST biotypes. A genotype link exists between old European, Australian, East African, Indian strains and those found in southern Africa today



on slaughterhouse inspection and isolation of mycoplasmas from suspicious lesions. The possible existence of an unknown *Mmm*SC reservoir or of low pathogenic strains that may revert to virulence needs to be studied extensively. MLST results will be compiled into a website that will allow regular follow-up on the emergence of new strain types through networking of veterinary laboratories involved in CBPP diagnosis.

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Presentation 6

Preparation of heat-tolerant CBPP vaccine

An initial assessment of the suitability of the xerovac process

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INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is an economically important disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* (small colony biotype) and is the only bacterial disease in the OIE List A diseases. In situations where eradication by stamping out cannot be implemented, vaccination offers an alternative method for control of the disease and this is commonly applied in areas where CBPP is still widely prevalent as in Africa.

The types of vaccines in current use are live attenuated broth cultures prepared using strain T1/44 or its streptomycin resistant derivative,T1/SR. Due to their short shelf life and heat lability liquid vaccines have now been abandoned in favour of freeze-dried products. However, although lyophilized CBPP vaccines are relatively heat resistant they still have to be conserved under cold storage up to use in the field. The need to maintain a cold chain adds additional costs to the usage of such vaccines.

The development of a system of vaccine dehydration that would yield a product with increased heat tolerance would reduce the extra costs involved in the maintenance of the cold chain in post-production vaccine handling. Recently a desiccation procedure for the preservation of rinderpest and *peste des petits ruminants* (PPR) vaccine was described. The method, which tries to mimic some of the survival strategies of cryptobiotic organisms, was shown to yield heat stable rinderpest and PPR vaccines. The method, which has come to be known as "xerovac", employs the disaccharide trehalose as the only preservative in the desiccation process. This article presents a summary of results obtained in trials aimed at assessing heat tolerance of CBPP vaccines prepared using the xerovac method.

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[3] AVIS College, London

MATERIALS AND METHODS

CBPP vaccine strain T1/44 grown in Gourlay's medium with or without trehalose was dehydrated in excipients containing varying concentrations of trehalose by the xerovac method. The resultant products were tested for heat tolerance by holding the vaccine at 45°C for two weeks. Residual mycoplasma content was determined at given time points by estimation of viable count in microplate titrations.

Portions of liquid vaccine cultures were also freeze-dried in a stabilizer consisting of skimmed milk or a mixture of skimmed milk and trehalose. Heat-resistance tests of the two types of preparations in comparison with a xerovac product were determined as described earlier.

A separate trial sought to find out if the addition of divalent cations in the dehydration excipient would increase the relative thermostability of the resulting vaccine.

Mycoplasmas in fluid vaccine culture were precipitated by addition of chitosan followed by slow centrifugation. Precipitated vaccine was dehydrated by the xerovac procedure and then subjected to heat stress.

RESULTS AND DISCUSSION

Effect of the addition of various trehalose concentrations

The effect of the addition of various trehalose concentrations into mycoplasma growth medium on the heat tolerance of the subsequent dehydrated product is shown in Table 6.1. These results suggest that the addition of trehalose in mycoplasma growth medium had beneficial effects not only in terms of the degree of titre loss upon dehydration but also on the degree of heat tolerance of the subsequent dried culture.

Effect of varying concentrations of trehalose as dehydration excipients

CBPP vaccine cultures that were prepared in medium containing 10 percent trehalose were dehydrated by the xerovac method in excipients composed of 0, 5, 10 or 25 percent trehalose. Heat resistance of dehydrated preparations were as shown in Table 6.2. The higher the concentration of trehalose in the dehydration stabilizer, the higher would be the heat resistance of the resulting dried mycoplasma culture.

Effect of divalent cations

Aliquots of the mycoplasmas harvest that were dehydrated in 25 percent trehalose containing 1.2 mM of either of the following aqueous solutions: copper sulphate, magnesium

TABLE 6.1
Dehydration of vaccine cultured in medium with 10 percent

% trehalose in growth medium	Titre of liquid vaccine	Day and titre at 45°C				% RM
		0	4	8	14	
0.0	9.6	6.2	0	0	0	3.07
0.5	10.0	7.3	0	0	0	4.67
1.0	9.8	7.0	0	0	0	4.25
2.5	10.1	7.2	3.4	0	0	4.24
5.0	9.5	8.3	5.3	0	0	3.95
10.0	9.3	8.3	6.3	0	0	3.64

sulphate, zinc acetate, calcium chloride or nickel chloride were subjected to heat stress at 45°C. The findings were as shown in Table 6.3.

The addition of the named ions into the dehydration excipient at the concentrations used was deleterious to *M. mycoides* subsp. *mycoides* and resulted in high losses of mycoplasma viability. In all but one case there was no detectable live mycoplasma by Day 3 of incubation at 45°C.

Comparison of freeze drying and xerovac processes

The trials carried out demonstrated the superiority of heat the tolerance effects of a vaccine prepared using the xerovac method in comparison with a product resulting from lyophilization for 56 h using the same concentration of trehalose (Table 6.4).

TABLE 6.2
Various concentrations of trehalose as the stabilizer

% trehalose	Titre of liquid vaccine	Day and titre at 45°C					% RM
		0	4	7	12	14	
0.0	9.0	6.4	5.2	2.7	0	0	2.86
5.0	9.1	8.1	6.7	6.4	6.0	6.0	2.21
10.0	8.9	7.4	7.2	6.9	6.3	5.8	2.29
25.0	9.0	7.5	7.3	6.6	6.7	6.7	1.55

TABLE 6.3
Effect of incorporation of various divalent cations into the dehydration excipient

Stabilizer type	Titre of liquid culture	Residual titre at 45°C on day				% RM
		0	3	7	14	
Trehalose+CuSO4	7.3	0	0	0	0	3.9
Trehalose+MgSO4	7.3	2	0	0	0	3.7
Trehalose+Zn acetate	7.3	3.3	1.7	0	0	4.3
Trehalose+CaCl2	7.3	3.7	0	0	0	3.8
Trehalose+NiCl2	7.3	2.1	0	0	0	3.4
Neat culture	7.3	4.4	0	0	0	4.2

TABLE 6.4
Heat stability of lyophilized and xerovac CBPP vaccine at 45°C

Stabilizer type	Titre of liquid culture	Residual titre and day at 45°C			
		0	3	7	14
10% trehalose FD	9.0	7.9	6.6	6.3	4.4
25% trehalose FD	9.0	7.9	6.5	5.6	4.3
4% skimmed milk FD	9.3	8.0	7.6	6.3	5.2
25% trehalose Xerovac	10.4	9.3	9.0	9.0	8.6

Effect of chitosan and results of extended heat stability tests of xerovac CBPP vaccines

The results obtained in the extended thermal degradation trials at 37 or 45°C are shown in Tables 6.5 and 6.6.

Heat-resistance tests over a longer period of time confirmed earlier findings that suggested that the higher the concentration of trehalose stabilizer the better the resistance to heat stress. Similarly, the use of chitosan to coerce the vaccine mycoplasma into precipitation surprisingly conferred extra heat protection to the subsequent dehydrated vaccine. However, relatively higher losses of mycoplasma were encountered on the xerovac vaccine precipitated using chitosan. This loss is thought to stem from the fact that mycoplasmas are fragile because they lack cell walls and it is therefore possible that increased fragility and damage could have arisen from the greater degree of mixing needed to homogenize the chitosan–vaccine precipitate.

CONCLUSIONS

- The experiments carried out show that rapid dehydration of CBPP vaccine in an excipient composed of a high concentration of trehalose following the xerovac method renders the product more heat tolerant than a similar freeze-dried vaccine.
- Addition of chitosan as a mycoplasma precipitating agent conferred additional heat resistance to the vaccine.
- Addition of divalent ions into CBPP vaccine dehydration excipient resulted in huge losses of mycoplasma viability during the xerovac drying process. The reasons for decreased

TABLE 6.5
Stability of CBPP xerovac at 45°C for 45 days

Vaccine stabilizer type	Titre of liquid vaccine	Titre and day at 45°C							
		0	3	7	10	14	20	30	45
5% trehalose	10.20	8.40	8.10	0	0	0	0	0	0
5% trehalose + chitosan	10.70	8.60	8.00	7.0	6	4.7	4.6	0	0
25% trehalose	9.80	8.70	8.40	7.7	6	6.2	6.1	6	5.8
25% trehalose + chitosan	11.40	9.50	9.30	8.3	8	7.7	7	6.7	6.4

TABLE 6.6
Stability of CBPP xerovac at 37°C for 45 days

Vaccine stabilizer type	Titre of liquid vaccine	Titre and day at 37°C							
		0	3	7	10	14	20	30	45
5% trehalose	10.20	8.40	6.5	6.4	5.8	3.8	3.1	0	0
5% trehalose + chitosan	10.70	8.60	8.5	8.5	7.7	6.8	6.5	5.9	5.7
25% trehalose	9.80	8.70	8.3	8.2	8.2	7.9	7.8	7.2	6.9
25% trehalose + chitosan	11.40	9.50	9.3	8.8	8.7	8.5	8.4	7.6	7.6

viability of the mycoplasmas are not clear but could possibly be attributed to osmotic shock and/or excessive decrease in pH.

- The xerovac dehydration process still needs to be improved further with a view to increasing the heat tolerance and hence shelf lives of the vaccines. It will, for example, be necessary to ensure that high initial mycoplasma titres are obtained in the pre-dehydration liquid vaccine cultures on account of the titre losses encountered during the drying process.

ACKNOWLEDGEMENTS

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Presentation 7

Development and validation of new technologies for control of CBPP in east Africa

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INTRODUCTION

Earlier meetings of the CBPP Consultative Group have identified the need for development and validation of new strategies, vaccines, diagnostic tests and antibiotics for control of CBPP in Africa (FAO, 2004; FAO, 2001). To address some of these needs, a collaborative research project was developed in 2003, involving the Moredun Research Institute (UK), the University of Bern (Switzerland), the International Livestock Research Institute (Kenya), the Kenya Agricultural Research Institute, the Animal Diseases Research Institute (Tanzania), VSF Germany and the Kenya Department of Veterinary Services. The aims of the project are (i) to determine whether a buffered formulation of the current vaccine provides better immunity in individual animals or at the herd level, and whether this additional protection is influenced by delivery through private or government teams; (ii) to estimate the social and economic impact of vaccination at the herd level; (iii) to determine whether there is an economically viable and sustainable alternative to the conventional vaccine distribution system; (iv) to evaluate diagnostic tests for diagnosis of CBPP; and (v) to develop new generation vaccines with increased efficacy and reduced post-vaccinal reactions and evaluate their safety and efficacy under laboratory conditions. The research project was been funded by the Wellcome Trust for a period of five years, and commenced in August 2005.

This report describes the project and presents results from the first year of the study.

SAFETY AND EFFICACY OF A BUFFERED VACCINE

Since 1956, the vaccine strain T1/44 – an attenuated live *Mmm*SC vaccine – has been used for control of CBPP, although safety and efficacy of the vaccine has been questioned (March, 2004; Thiaucourt *et al*., 2000; Rweyemamu *et al.*, 1995). During the 2003 meeting of the CBPP Consultative Group, March recommended a modification of the T1/44 vaccine (March, 2004). The modification was based on addition of a buffer system (HEPES) to the growth media of the vaccine strain, addition of a pH indicator to the reconstitution fluid and the use of phosphate-buffered saline (PBS) for reconstitution of the freeze-dried vaccine in the field. The recommendation was based on observations that pH in both growth

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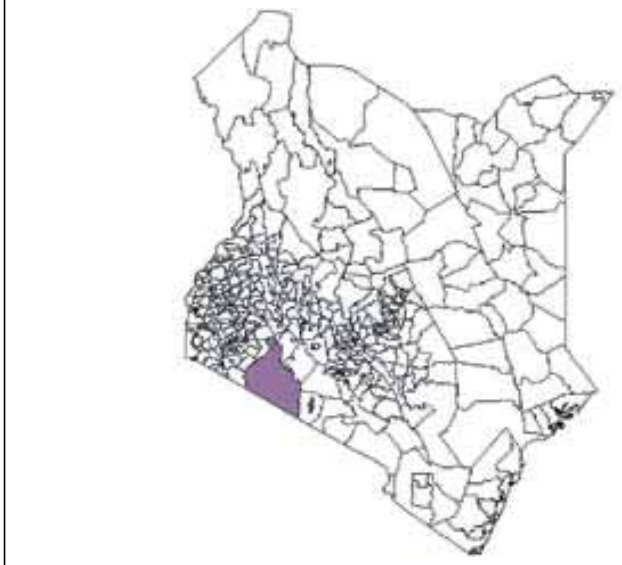
media and reconstitution fluid can reach levels suboptimal for survival (Waite and March, 2002) and vaccine efficacy (Pollack *et al.,* 1969) of *Mmm*SC.

To compare safety and efficacy of the buffered vaccine with the currently used vaccine, the study includes a double-blinded experimental trial and a field study in the Maasai ecosystem of southern Kenya. The experimental trial is being conducted at the Kenya Agriculture Research Institute, Nairobi. For the trial, 90 cattle are vaccinated either with the buffered vaccine, the currently used vaccine or a placebo. Half of each of the cohorts will then be challenged intrabronchially after two and twelve months, respectively, with a field strain of *Mmm*SC. Results of this study will indicate whether the vaccine composition influences development of site reactions (safety), seroconversion, infectivity, infectiousness, morbidity or mortality (efficacy). In a supplementary trial to evaluate the use of a bronchoscope for challenge, 16 additional cattle were infected in May 2006. In line with earlier studies (Thiaucourt *et al*., 2000) the challenge was only moderately successful, with five cattle developing neither symptoms nor lung lesions.

The field study is currently being conducted in three subdistricts of the Narok district in south-west Kenya bordering Tanzania (Figure 7.1). The study area is semi-arid and is used predominantly by (semi)-pastoral Maasai with mean herd sizes of 200 cattle. The area has been classified as Zone IV, CBPP infected area with intensive annual vaccination, active screening and strict cattle movement (Department of Veterinary Services, Kenya). The study consists of a participatory epidemiology study, a cross sectional survey and a cohort study.

The participatory epidemiology study was conducted in May 2006, and provided information on distribution and socio-economic impact of CBPP and other diseases in the study area. In agreement with earlier studies (Mariner and Catley, 2004), CBPP was well known among pastoralists and consistently ranked within the five most important livestock

FIGURE 7.1
Map of Kenya: shaded area shows study area for field trial



diseases. During the participatory study clinical signs of CBPP were found in two different herds, and CBPP was later confirmed using c-ELISA in both herds.

The cross-sectional study was conducted in August 2006 with the aim of estimating between-herd prevalence in the area, and to collect further data on the socio-economic impact of CBPP and risk factors for herds being seropositive. The study population consisted of approximately 6 400 cattle from 160 randomly selected herds. Out of the 160 herds only one appeared to be affected by CBPP at the time of sampling, but infection has not yet been confirmed. Data and blood samples collected during the cross-sectional study are currently being analysed. For estimation of sero-prevalence, CFT and c-ELISA tests will be used and, in line with recommendations of OIE (OIE, 2000), any herd will be designated positive if one or more animals react positive in at least one of the two tests (parallel interpretation). Because sensitivity and specificity of the tests in parallel interpretation are not known, a test evaluation will be conducted.

The cohort study will start at the end of 2006, and will comprise two yearly vaccinations of approximately 90 000 cattle in the study area. For each vaccination, several livestock crushes in the area will be staffed either by a government or a private vaccination team to test whether differences in efficacy and safety of the two vaccines are influenced by the vaccine delivery system. All cattle from individual herds coming to the crushes will be randomly allocated to either of the two vaccines, so that half of the cattle in each herd receives the buffered vaccine and the other half receives the currently used vaccine. Both the administration of the vaccines and the evaluation of safety and efficacy will be carried out "blinded". A subsample of vaccinated animals will be tested pre- and post-vaccination for titres of antibodies (CFT and c-ELISA) and the presence of antigen. The subsample will additionally be followed up for comparison of vaccine site effects. Using a network of community animal health workers, all vaccinated plus 500 control herds will be monitored for signs of CBPP during the follow-up period of two years.

SOCIAL AND ECONOMIC IMPACTS OF VACCINATION

In view of the relatively low morbidity and mortality of CBPP in endemic areas and high costs of vaccination campaigns, Thomson proposed that "the expenditure [for blanket vaccination campaigns] is, at best, only possibly cost-beneficial" (Thomson, 2005). To estimate social and economic impacts of vaccination in this study, both a traditional socio-economic framework leading to cost–benefit analysis (CBA) and a contingent valuation method (CVM), which will evaluate the willingness to pay (WTP) for the vaccine against the demographic characteristics of households, will be used. Data for the evaluation will come from the participatory epidemiology study, from several questionnaire surveys during the cross-sectional and the cohort study and from follow-up of a sub sample of vaccinated and control herds.

ALTERNATIVES TO THE CONVENTIONAL VACCINE DISTRIBUTION SYSTEM

Distribution of CBPP vaccines in Africa can be negatively affected by financial, geographical and political restraints (Musisi *et al*., 2004). Alternative systems to state-monopolized distribution, which can flexibly address demands for vaccination and constantly ensure high-quality vaccination covering larger areas, could therefore help in controlling CBPP. Results from the socio-economic and vaccine efficacy studies from the first two vaccinations and an

additional survey of service providers will be used to develop an alternative vaccine distribution system, which will most likely be based on a public–private partnership with some kind of cost-recovery. To evaluate this alternative vaccine distribution system, the third vaccination of approximately 50 000 cattle will be commissioned. Once again, vaccination will be followed up to determine safety, efficacy and economic sustainability.

Both the relatively low vaccine coverage in the first two vaccinations (approximately a quarter of the population will be vaccinated) and the reduced study population in the third vaccination will help to prevent a large proportion of herds becoming susceptible to disease within a short period of time after the project is complete.

EVALUATION OF DIAGNOSTIC TESTS FOR DIAGNOSIS OF CBPP

Successful estimation of vaccine efficacy depends heavily on correct classification of diseased (vaccine failure) and non-diseased animals among vaccinates (Plikaytis and Carlone, 2005). Several diagnostic tests have been evaluated to classify animals (e.g., Geiger, 2004; Bellini *et al.*, 1998), but the performance of these tests in vaccinated animals or when interpreted in parallel seem unclear. Other diagnostic tests have been developed recently (Bruderer *et al.,* 2002; Gorton *et al.,* 2005) but have not yet been validated, while methods for validation of diagnostic tests have improved (Enøe *et al.,* 2000, Georgiadis *et al*., 2003). Evaluation of diagnostic tests in our study will focus on the sensitivity and specificity of an (antigen detection) latex agglutination test (LAT), an LppQ-ELISA and a Taq-Man PCR, their performance in vaccinated animals and the establishment of cost-effective strategies for their use in individual animal diagnosis, herd diagnosis, prevalence estimation and surveillance programmes. Test evaluation will be based on samples from the experimental study, from the field evaluation of the vaccines and from an additional slaughterhouse survey.

EVALUATION OF IMMUNOLOGICAL RESPONSES TO VACCINATION

Development and validation of improved vaccines will entail a better understanding of pathogenesis of the disease and the evolution and maintenance of host immunity to natural infections (Tulasne *et al*., 1996; Abusugra *et al.*, 1997). Evaluation of immunological responses to vaccination in our study aims to compare immune responses engendered by the current and buffered T1/44 vaccine under laboratory conditions and to generate T-cells reagents from immune cattle following challenge for use in validating empirically identified antigens. The evaluation will be based on samples from the experimental study and purified antigen from different sources.

DEVELOPMENT AND VALIDATION OF NEW GENERATION VACCINES

Currently used vaccine strains (both T1/44 and T1/SR) have several disadvantages: they can give rise to severe post-vaccinal reactions (Sori, 2005); vaccine efficacy is waning and probably leaky (Thiaucourt *et al.*, 2000); and residual virulence in the vaccine strain can induce clinical CBPP (Wesonga *et al.*, 2004). Further attenuation is required to reduce post-vaccinal reactions and the virulence of vaccine strains. An important virulence factor of *Mmm*SC, which may be responsible for the site effects, has been identified as a metabolic enzyme (glycerol-3-phosphate oxydase, GlpO) (Pilo *et al.,* 2005). This factor is also recognized as

antigenic by the immune system. Lipoprotein Q (LppQ) is another antigen of *Mmm*SC that is recognized by the immune system, and studies by Nicholas *et al.* (2004) have suggested that immunization with this factor may result in exacerbation of pathology upon challenge.

To reduce virulence and preserve antigenic activity, a new generation attenuated vaccine is being constructed at the University of Bern under the auspices of the project. The construct will be a mutant of *Mmm*SC strain T1/44 with a deletion mutation for the LppQ gene and an additional mutation of the GlpO gene that results in inactivation of the enzyme. This attenuated mutant will have the additional benefit of being a marker (DIVA) vaccine if used in conjunction with the LppQ ELISA. To date, the study has shown that the LppQ gene is present in only one copy in the vaccine strain T1/44 and other field strains of *Mmm*SC (Bischof *et al.,* 2006), and an LppQ$^-$ knockout mutant of MmmSC strain T1/44 has already been generated.

Effects of the LppQ$^-$ knockout mutant will be studied *in vivo* under laboratory conditions at the Kenyan Agriculture Research Institute. The objectives of these studies will be to compare the LppQ$^-$ knockout mutant and the currently used vaccine in terms of humoral and cellular immunogenicity and to estimate the safety and efficacy of the LppQ$^-$ knockout mutant and evaluate the immunogenic effects of vaccination with purified recombinant LppQ.

TRAINING AND KNOWLEDGE TRANSFER

The study has an extensive training component: three PhD and six MSc students from Tanzania and Kenya as well as a PhD student from Colombia will be trained during the project. Upon completion of the study its results will be disseminated using a workshop, a series of leaflets printed in Kiswahili and Kimaasai, project reports and publications in scientific journals. Additionally, information will continuously be disseminated to end users.

Further information about the project may be sought at: http://www.moredun.ac.uk/cbpp

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Presentation 8

The effect of Cyclosporin A (CsA) on CBPP development

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Contagious bovine pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC) is one of the most important diseases in Africa where disease control is effected by vaccination. In the African context, movement control of animals as well as slaughter and compensation strategies, which worked well in some countries, are not applicable owing to financial constraints and century-old transhumance practice.

However, presently, only two vaccine strains are accepted for vaccine preparation by the Pan African Vaccine Centre (PANVAC): T1/44 and T1/SR. Both strains have been developed empirically and have served their purpose to some extent. While T1/44 is incriminated for serious side effects in vaccinated cattle and may cause outright disease if applied intratracheally, T1/SR is eliciting a poor immune response which lasts a relatively short period of time. Further inactivated (saponin inactivation) vaccines, split vaccine in ISCOM constructs (immune stimulating complexes) as well as lipoproteins in an ISCOM construct exacerbated the disease after exposure of vaccinated cattle to infected animals.

All these observations made it clear that the research in vaccine development is imperative in order to control this disease. However, vaccine research should be based on a clear understanding of the pathogenesis of CBPP which is missing to date. The role of innate or acquired cell mediated and humoral immunity in conferring protection against the *Mmm*SC has not yet been elucidated. On the other hand, the pathological lesions caused by the aetiological agent have been considered indicative of an immunopathological process.

As a first step in this direction a study using Cyclosporin A as an immunosuppressant agent was initiated. The drug is a suppressor of the immune response related to the T-cell system.

In this study ten naive cattle were exposed to in-contact infection with animals infected by intubation with a strain of *Mmm*SC. Clinical symptoms, antibody response, IFNγ release and pathological changes at post-mortem examination were analysed and compared with the events following in-contact infection of an equal number of animals kept under daily treatment with Cyclosporin for the entire observation period of 84 days. The daily dosage of 4 mg CsA/kg body weight has been selected as the upper ceiling concentration that could safely be applied intramuscularly in cattle. We were, however, aware of the fact that oral dosage in monogastric animals is substantially higher, yet the ruminant stomach system would not allow *per os* application of CsA.

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Under the conditions of the experiment, Cyclosporin appeared to condition the pathogenesis of CBPP by delaying the events that follow infection, supporting further that the immune response may have an impact on the disease outcome. The study presented further emphasizes again the need for continued research into the pathogenesis of CBPP as only a detailed comprehensive knowledge of pathogenesis will allow the formulation of an effective vaccine suitable for long-term control and eradication of CBPP.

The results presented in this paper are described in more detail in a publication entitled: Clinical, humoral and IFNγ responses of cattle to infection with *Mycoplasma mycoides* var. *mycoides* small colony and attempts to condition disease pathogenesis. *Onderstepoort Journal for Veterinary Research* (Accepted).

Presentation 9

Control of CBPP in Africa

Results of the First Research Coordination Meeting in Windhoek, 30 October to 3 November 2006

H. Unger[1]

INTRODUCTION AND BACKGROUND

Despite numerous efforts, CBPP continues spreading in Africa. A Coordinated Research Project (CRP) on monitoring CBPP in Africa initiated in 1998 by IAEA helped to gain valuable information on the performance of a c-ELISA and latex agglutination tests, but did not succeed in terms of improving control strategies applied in the field. Since the start of this CRP, a number of new facets in immunology, pathogenicity and host/pathogen interactions were described and new approaches like the antibiotic treatment for "sterilizing" herds were evaluated. Nevertheless a number of key factors are still hampering the development of efficient tools to combat the disease in the African setting.

The new CRP on Control of CBPP in Africa is focusing on the early detection of *Mycoplasma mycoides* small colony (*Mmm*SC) in the field, validating the existing serological tools for fitness for purpose and aims at applying the existing knowledge in cellular immunology to define the cattle status as "chronically infected" and "protected".

Veterinarians from 11 cooperating countries in Africa took part in the first Research Coordination Meeting (RCM) and presented the disease situation and interventions. The major constraints identified as hampering control of the disease were:

- Uncontrolled cattle movement; leading to the spread of CBPP.
- Treatment of affected cattle by livestock owners lacking confidence in vaccination.
- Lack of technical means to quickly diagnose CBPP and quantify the prevalence by veterinary services.
- Need of a sensitive, specific and quick "field test" for early detection.
- Long duration of vaccination campaigns to show impact.
- Inability to monitor protection after vaccination.

The last CRP on monitoring CBPP helped to improve the c-ELISA kit and produced a number of valuable results. The experimental latex-agglutination tests gave inconclusive results and due to problems with quality assurance were not developed further.

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Concerning the scientific background to CBPP it was felt that:

- Too few pathogenicity factors are identified so far to clearly describe the pathology.
- Protection mechanism and protective antigens are still unclear.
- Spreading parameters in herds and populations are not well defined.
- Diagnosis specifically for latent carriers must be improved.

Responding to these shortcomings the following work programme was initiated:

- Evaluation of the serological tests for fitness for purpose (c-ELISA, LPPQ-ELISA).
- Introduction of isothermic PCR (LAMP) for the early and specific detection of CBPP.
- Evaluation of tools and genetic markers for molecular epidemiology of CBPP and their potential use in control programmes.
- Evaluation of skin testing for the detection of infected and carrier animals (expression antigen, LPPQ).

EVALUATION OF THE SEROLOGICAL TESTS FOR FITNESS FOR PURPOSE

The last CRP produced limited results on the c-ELISA, as only ten plates were handed out to each participant. The diagnostic specificity of the c-ELISA was 99 percent and the diagnostic sensitivity ranged around 90 percent in infected herds displaying clinically sick animals. The first positive results were obtained four weeks after infection and could be detected for at least four to six months. Vaccinated animals were detected by c-ELISA for up to three months after the application, but only in a limited number of animals (~10 percent in T1/44 vaccinated and ~23 percent in T1/SR vaccinated animals). The number of c-ELISA positive animals was always higher in the c-ELISA than in the complement fixation test (CFT), compromising the relative sensitivities.

As CFT and c-ELISA are measuring different antibody classes, these results do not come as a surprise. The CFT is prone to producing false positive results and due to its low sensitivity produces only reliable results until three months after infection. So the "real" number of responders in CFT-tested infected herds is certainly not a gold standard. This is why the new CRP is now embarking on correlating the results of the LPPQ ELISA with the c-ELISA and post-mortem, when possible.

One of the expected results is defining the response to vaccination. Apparently, the LPPQ test does not detect vaccinated animals, which would be very useful in control programmes applying vaccination.

In order to define the cut-off in correlation to the diagnostic sensitivity and specificity, the ELISA results will be analysed by receiver operating characteristics (ROC) to define specific settings for a given purpose.

As the last validation exercise showed shortcomings in robustness and ruggedness, the kits as well as the specimens will be subjected to temperature stress in order to define the limits of transport and handling.

EVALUATION OF LAMP PCR FOR THE EARLY AND QUICK DIAGNOSIS OF CBPP

The application of polymerase chain reaction (PCR) capable of a rapid, sensitive and highly specific diagnosis of CBPP has been published but was never applied in veterinary laboratories as a standard test procedure. This is partly due to problems with sampling and transport of samples, but as well to lack of equipment, reagents and training. Due to the complex

techniques involved, it can only be performed in well-organized laboratories. In order to utilize the benefits of PCR a new isothermic method, named loop-mediated isothermal amplification (LAMP), was developed together with the Institute of Veterinary Bacteriology, University of Berne. This test system has the advantage of a quantitative analysis of the samples for DNA of the pathogen without the need of a thermal cycler (*www.loopamp.eiken.co.jp/e/lamp/index.html*). The reaction can easily be done in a water bath of 65°C and read after 30 to 60 minutes by eye (milky precipitates from magnesium pyrophosphate) or after adding Sybrgreen by a colour change from orange to green in a positive case. This allows performance of this test in the field. To quantify the amplification results, a turbidimeter can be used. Such instruments are under evaluation.

The CRP will work on sampling procedures, DNA extraction techniques and the correlation of the LAMP results with culture and real-time PCR.

LAMP PCR can be performed in the field, and the vials with the amplification product can be sent to the next laboratory for further molecular testing, not being destroyed by heat or contamination, which is a significant advantage over other existing methods. At a later stage, LAMP readers will enable the field staff to perform a quantitative analysis in the field and transmit the data by GMS to a central veterinary laboratory to initiate an early response.

These samples will also be important for the molecular epidemiology component of this CRP. The advantage lies in the fact that the sample does not need to be cultured for the subsequent steps, which reduces the risk that the culture media influence the genetic profile.

MOLECULAR EPIDEMIOLOGY OF CBPP AND THEIR POTENTIAL USE IN CONTROL PROGRAMMES

Recent publications showed clear genetic differences between African and European strains of *Mmm*SC or differences in expression profiles (Poumarat, 1995). These differences can partly be related to pathogenicity (Vilei *et al.*, 2000). Research into additional antigenic and genetic markers is continuing and creates the potential to identify differences in isolates which might be related to the origin of an outbreak. Multi-locus sequencing, randomly amplified polymorphic DNA and restriction fragment length polymorphism are the techniques to be evaluated (Lorenzon *et al.,* 2003). Data created will not only help in the understanding of the epidemiology of CBPP but equally benefit the development of new vaccines and diagnostic tests.

Skin testing for the detection of infected and carrier animals

Resistance to CBPP is conferred by the cellular immune system. This has been apparent since the development of live vaccines against this disease. Reports indicating a strong correlation of a CD4+ response and survival of infection corroborate this finding. Lymphocyte stimulation assays employing mycoplasma antigen did not consistently elicit an interferon response as in mycobacterial infections. As TNFα expression is stimulated by *Mmm*SC in alveolar macrophages, this should be as well the case in Langerhans cells, which are potent antigen-presenting cells of the dendritic cells lineage in the skin and affect mast cells, which increase vascular permeability through the release of vaso-active amines leading to delayed type hypersensitivity (DTH). This increases the skin thickness after 24–96 hours (Pollock *et*

al., 2003). Windsor showed in experimentally infected animals that a positive skin reaction to a membrane antigen correlates with infection under control, i.e. no clinical signs (Windsor *et al.*, 1974). If skin positives do show a positive CFT titre, they still incorporate live Mycoplasmas in quantities correlated to the titres observed. Experiments employing an expression antigen of *Mycobacterium bovis* as a skin test showed that such an antigen has to be employed in large quantities (400 µg/dose) or it has to be adjuvant formulated (Whelan *et al*., 2003). The only CBPP specific antigen available is LPPQ. This lipoprotein certainly has major effects in the pathogenesis of CBPP, eliciting a strong antibody response and possibly exacerbating the disease (Nicholas *et al.,* 2004). This expression antigen will be employed in a skin test as a pure formulation to identify toxic effects. When tested negatively, a toll-like receptor stimulant will be added to specifically target the cellular immune system and to elicit a skin reaction (Spohn *et al*., 2004). Such a skin test could be worthwhile in controlling cattle for CBPP along the trade and grazing routes and might result in more targeted control strategies including the individual culling of seropositive reactors.

CONTROL OF CBPP BY VACCINATION

One of the control strategies for CBPP is vaccination with a freeze-dried live attenuated T1-strain vaccine. The vaccination should elicit an immune response of the cellular type, which is thought to confer protection. The original T1 strain still expressed the PTSG gene leading to hydrogen peroxide production and specific Willem's reaction. More attenuated strains T1/44 and T1/SR have a very much reduced induction of PTSG and only seldom lead to this reaction (Garivaud *et al*., 2004). Interestingly, reports from the 1960s and 1970s showed that only a few animals seroconverted and produced positive CFT titres. Contrary to this, roughly 70 percent of vaccinated animals seroconvert in CFT. Historic reports describe duration of protection of around two years, while today protection by vaccination is questionable as the ever-expanding map of affected countries shows. Of course, vaccination is not carried out at the same intensity as during the Pan African Rinderpest Campaign (PARC) and even then a different vaccine formulation was used – the combined Rinderpest and CBPP vaccine [Bisec]. The "old" vaccines were delivered as cold-stored broth at a maximum titre while today, because of freeze drying, a loss of around 2 logs of live organisms is experienced. This certainly shifts the immune response of the immunized to a B-cell type, which might not be as protective and maybe even deleterious as in this way macrophages will easily engulf the pathogen as described for *Mycoplasma. ovipneumoniae* (Al-Kaissi and Alley, 1983). The fate of *Mmm*SC in macrophages is not yet documented, but Howard *et al*. (1976) showed that *Mycoplasma dispar* and *Mycoplasma agalactiae* subsp. *bovis* survived and replicated in bovine alveolar macrophages under specific culture conditions.

As research into new vaccines is ongoing, it will be important to define the protective mechanisms against CBPP. Serological methods will not play a major role in this respect, but the skin test and a simplified lymphocyte stimulation assays might be of assistance.

CONTROL OF CBPP BY TREATMENT

Preliminary results of treatment experiments and epidemiological models show economic advantages in the use of antibiotics in infected herds. Taking this as a baseline for a control strategy, the diagnosis of CBPP has to be more rapid. The application of the LAMP PCR will

be valuable for identifying excreting cattle and seromonitoring for assessing the infection status of the herd.

CONCLUSIONS

The CRP on control of CBPP is set to support member countries and also the scientific community with validated and new tools in the fight against CBPP. Only the wide application of these techniques will show their potential benefit to our understanding of the pathophysiology of CBPP and in the fight against this disease.

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Presentation10

The evolution of CBPP in southern Africa: 2004–2006

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INTRODUCTION

The importance of contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subspecies *mycoides* small colony (*Mmm*SC), to southern Africa was acknowledged by the Chief Veterinary Officers (CVOs) of SADC at their workshop in July 2003 in Pretoria. The CVOs then noted that the CBPP-affected countries were Angola, northern Namibia, Tanzania and Zambia and those at immediate risk were neighbouring countries, namely Botswana, Malawi, Mozambique, the rest of Namibia and Zimbabwe. The CVOs recommended two phases to tackle the CBPP problem in the region, namely an emergency phase and a recovery phase. The emergency phase would tackle the two CBPP epidemiological clusters: the southern Angola–western Zambia–northern Namibia cluster and the southern Tanzania–north-eastern Zambia cluster. The emergency phase would address emergency vaccination of all cattle in the affected areas, access to laboratory diagnosis, heightened surveillance and cattle movement control, public awareness and elaborate strategies that are practical and realistic to convince politicians to invest in eradication of the disease. This paper focuses on the recent–current CBPP occurrence and control activities while at the same time highlighting some of the successes achieved and the challenges the countries in the region face to eliminate CBPP.

OBSERVATIONS ON THE EVOLVING CBPP REGIONAL SITUATION

Angola

Historical perspectives: CBPP has been in Angola for a very long time and it has been the primary source of infection for the neighbouring countries, mainly Namibia and Zambia. The prolonged civil strife that Angola experienced in the 1970s through to 2000 made control virtually impossible.

Recent–current situation: With the return of peace to the country, efforts are under way to rebuild the various infrastructures that should enable the control of CBPP and

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other major diseases in the country. A central laboratory has been constructed in Luanda and is in the process of being equipped. Mobile veterinary laboratories have been sent to selected areas in southern Angola to enable provision of simple basic veterinary laboratory back-up and sample collection for transmission to better-equipped laboratories. In addition, sera samples are being collected from selected areas of the country to determine levels of exposure to CBPP and FMD.

Namibia

Historical perspectives: CBPP have been confined to the northern part of Namibia north of the veterinary cordon line for a long time. The highest upsurge in CBPP detection in recent times was in 1998, after which detection of the disease has declined remarkably.

Recent–current situation: In the Caprivi region, CBPP was detected in August 2003 following the illegal introduction of an infected animal in one *kraal* at the Maunga crush-pen area. The immediate source seems to have been Zambia, although it has not been established whether Zambia was the primary or secondary source. This was the first time Caprivi experienced CBPP in about 60 years. The disease subsequently spread to adjacent *kraals* in Batubaja and Mbilajwe crush pen areas since the cattle population there was naive with no routine vaccination (see Figure 10.1).

At Mukisa crush pen about 8 km from Katima Mulilo, 15 cattle were affected and were slaughtered in April 2004. Another primary focus of CBPP was detected in December 2004 at Kazuka crush-pen area in the Eastern Flood Plain. The areas affected by the disease to

FIGURE 10.1
Map of Namibia indicating approximate locations of outbreaks

date are Maunga, Batubaja and Mbilajwe crush pens. Serological evidence of infection using CFT has been detected at Samutetesi crush pen, which is approximately 10 km from the border with Botswana. Kazuka crush pen area is about 30 km from Botswana and 10 km from the Zambian border. The control measures in place include vaccination, depopulation and surveillance.

Following the detection of the disease a vaccination campaign was launched in October 2003, which covered 32 crush pen areas from Lianshulu to Gunkwe on either side of the outbreak area. Subsequent vaccinations were carried out in July/August 2004. Thereafter, annual vaccinations have been carried out. Compliance by farmers is good, despite some reactions caused by the vaccine. Vaccination coverage per session ranges from 96 percent to 98 percent. Vaccine reactions have been observed in cattle being vaccinated for the first time and owing to poor vaccination technique.

Namibia has not implemented compulsory slaughter in affected areas owing to an absence of enabling legislation and compensation. Farmers are being advised to slaughter affected cattle through the quarantine system. Initially, there was resistance to slaughter but later farmers realized that it was a better option. To date, some 1 200 cattle have been slaughtered from the previously affected areas in the south-western focus. At Kazuka some 129 cattle have been slaughtered voluntarily.

The crush-pen areas affected and those adjacent areas (i.e. Kapani, Samulandela, Samutetesi, Malengalenga, Kazuka, Itomba, Sigwe, Muzii and Sigwe) are under quarantine and the only cattle movement allowed is for slaughter by motor transport.

Following the detection of the disease, surveillance was intensified in the risk areas. Community based animal health workers (CAHWs) and farmers were sensitized of the disease. Surveillance is implemented throughout the region by the network of animal health technicians (AHT) and CAHWs. Surveillance is a combination of passive and active. Cattle are observed for clinical signs of disease during vaccination programmes, at quarantine farms and during outbreak investigation. There is also post-mortem examination at the abattoirs and other slaughter facilities. Sero-surveillance is being implemented in and around the outbreak areas.

In a sero-survey carried out in and around the affected areas (ten crush pens), evidence of exposure was only detected in the previously affected crush pens of Maunga, Batubaja, Mbilajwe and Samutetesi. Fifteen kraals had serological positive cases from 63 kraals tested. Positive animals per kraal ranged from 7 to 36 percent. The sero-surveys are ongoing and the results are being communicated to the farmers. Veterinary services staff with assistance of cooperating partners and CAHWs perform the disease surveillance. This is an ongoing exercise during scheduled vaccinations, at quarantine stations and in response to farmer reports.

Regarding surveillance and control of CBPP in Caprivi, the strategy that is being adopted by the veterinary services in east Caprivi is a combination of annual mass vaccinations of all cattle in the region (coverage has been over 95 percent, with good community collaboration), quarantine, movement control, surveillance and voluntary slaughter.

With regard to movement of cattle across the border, the DVS, Customs, Immigration, Security Forces and other stakeholders hold regular meetings with communities along the border with Botswana. At these meetings, the issue of cattle movements across the border

is mentioned to the farmers. Similarly, there is an annual cross-border meeting with Zambia. It has now been agreed with the Zambian veterinary authorities to harmonize vaccination campaigns in Caprivi with those in Sesheke and in the west Kazungula districts in Zambia, and to implement surveillance and animal identification by district through branding and monitoring of animal movement.

Some success stories: In northern Namibia, Cunene, North Central and Kavango regions cattle face threat of CBPP from the Angolan cattle; Namibia adopted continuous vaccination using T-44 annually since 1997 with 80–90 percent coverage. This has led to a reduction of number of cases detected from 200 to 5 annually.

In the Kazuka outbreak some 79 animals have died to date. Since the beginning of the outbreaks, about 600 cattle have died and mortality in the affected *kraals* ranged from 10–90 percent. Since April 2004 disease prevalence dropped to less than 1 percent in the previously affected areas.

The farming community is sensitized through meetings at Village Development Committee (VDC) level, at farmers' association meetings, Regional Development Coordination Committee (RDCC) level and through the electronic and print media. Farmers give feedback through reports at VDC meetings and at local DVS offices. Disease reports are submitted weekly to the Epidemiology Section at Veterinary Head Office, which reports regionally (SADC), and internationally through the World Organisation for Animal Health (OIE) system.

Tanzania

Historical perspectives: The first documented outbreak of the disease in the country was in 1916 and this occurred in the Loliondo division on the northern border with Kenya. Due to the veterinary services being rudimentary then and World War 1, the disease spread freely and by 1932 the area from Tanga to Mara on the eastern shores of Lake Victoria was infected. Concerted efforts to control the disease including strict animal movement control, and vaccination using Kabete broth vaccine enabled elimination of the disease by 1946 from all areas that were infected with the exception of a few pockets close to the border with Kenya.

A second outbreak occurred in the Loliondo division at Nguserosambu in 1955. Rigorous similar control measures were applied and the disease was contained by 1964, allowing a lifting of quarantine imposed following the outbreak in 1965; but vaccinations continued until 1974.

After an absence of about 25 years, the current raging outbreak started in 1990 at Soit Sambu again in the Loliondo division. It is believed bulls purchased from a neighbouring country for breeding purposes were the source of the disease. The disease spread to the Serengeti and Tarime districts in the Mara region through cattle rustling and from there to other parts of the country. In 1992 a new focus of the disease unrelated to the one in Loliondo occurred in the Kagera region. It was established that the outbreak originated from the neighbouring Rakai district in Uganda. The disease spread south of the Central Railway Line in 1994 and this was the first ever occurrence of CBPP south of the Central Railway Line. In these new areas, because CBPP was unfamiliar to many livestock workers,

it was mistaken for East Coast Fever or other pneumonic diseases. From 1995 onwards the disease has spread to many parts of the country (see Figure 10.2).

Recent–current situation: Since the 1990 outbreak, the Government of Tanzania has carried out several measures aimed at controlling the disease. The control strategy is based on the principle that mass vaccinations properly instituted over an extended period can reduce the disease incidence to an extent that other measures such as test-and-slaughter can be used to eradicate the disease. Whenever the disease occurs, quarantine is imposed in the affected area to prevent animal moving out or into the area. However, enforcing these quarantines has been difficult because of the vastness of the country and lack of resources.

A Presidential Circular issued in 2002 prohibited the inter-regional, long-distance trekking of trade stock. This made it mandatory to transport trade animals using vehicles, train wagons and/or ferries. To further enhance control of animal movement, in 2006 the government established a Directorate of Animal Identification and Traceability within the Ministry of Livestock Development (MLD). Currently, the most challenging cattle movement is that by migrating pastoralists (see Figure 10.3).

Some success stories: Notwithstanding the enormous challenges noted above, Tanzania has a number of success stories worth noting.

Maintenance of CBPP freedom in Zanzibar: The control measures instituted on the Tanzania mainland have to date prevented the spread of CBPP to Zanzibar, where the disease has never been recorded despite Zanzibar being heavily dependent on regular imports of slaughter animals from the mainland.

CBPP uninfected regions of mainland Tanzania: Two regions, namely Kilimanjaro and Lindi, have not reported the disease since the current CBPP incursion in 1990. The reasons for this are that the main animal husbandry practice in Kilimanjaro is zero grazing and there is low livestock population in Lindi. Apart from these, vaccinations in other areas coupled with quarantines imposed in areas where CBPP has occurred have managed to prevent its spread into these regions.

FIGURE 10.2
CBPP outbreaks, Tanzania

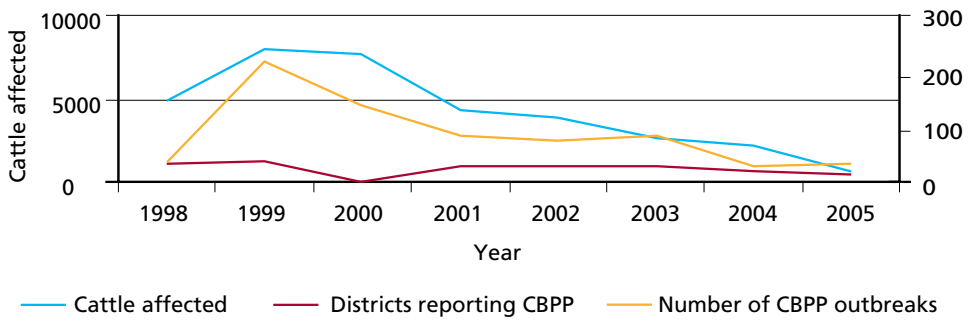
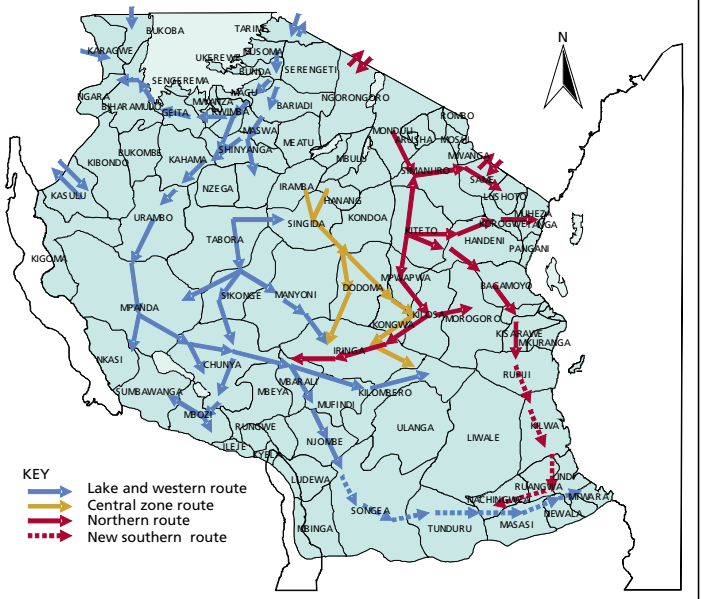


FIGURE 10.3
Map showing cattle movements associated with pastoralists in Tanzania



Southern highlands – impact of current control measures: The government objectives in the southern highlands were to stop the southward spread of disease to neighbouring countries and to reduce the incidence of CBPP in the area. Overall, the spread of CBPP has been arrested; the incidence of disease has declined; livestock farmers and other stakeholders are now aware of the disease and are more willing to participate in control measures being taken.

There has been no evidence of spread to Malawi, which has been verified by regular border surveillance in Malawi. Similarly, border district surveillance in Zambia has returned negative results. In addition, there are regular cross-border meetings of veterinary personnel in the border districts of Malawi, Tanzania and Zambia. There is also collaboration in cross-border monitoring of animal movements.

Slaughter facility surveys in the southern Tanzania districts, some of which border Malawi, and Zambia, show a steady decline on returns for CBPP lesions (see Figure 10.4). There has also been a sharp decline in the incidence of detected lesions in the region. Only 24 out of 3 927 serum samples (i.e. 0.6 percent) collected in 2005 from Mbarali, Mbozi, Sumbawanga and Mpanda districts in the project area tested positive.

The Kagera experience for CBPP elimination: CBPP was introduced in the Kagera region from the Rakai district in neighbouring Uganda in 1992. Tanzania was able to eradicate the disease in the region and by 1997 no new cases were being reported (see Figure 10.5). The main methods used in Kagera were:

- **Intensive vaccinations:** The region vaccinated all animals using T1/44 strain strictly as per the recommended regimen. Three vaccination rounds were conducted at three-month intervals in one year.

- **Slaughter of cases:** Vaccination was followed by intensive surveillance to detect the disease and eliminate animals that were found to have the disease. These animals were slaughtered and the meat was sold.

The reasons for successful CBPP eradication in Kagera are that (i) there was a well-trained and dedicated cadre of veterinary personnel that could closely supervize the vaccination exercise; (ii) most animals affected by a slaughter policy belonged to the National Ranching Company (NARCO) which is government owned: therefore, compensation was not a requirement. Furthermore, selling the meat assisted in offsetting the losses that would otherwise compromise the ranching operations; (iii) resources were made available

FIGURE 10.4
CBPP report in Southern Highlands of Tanzania

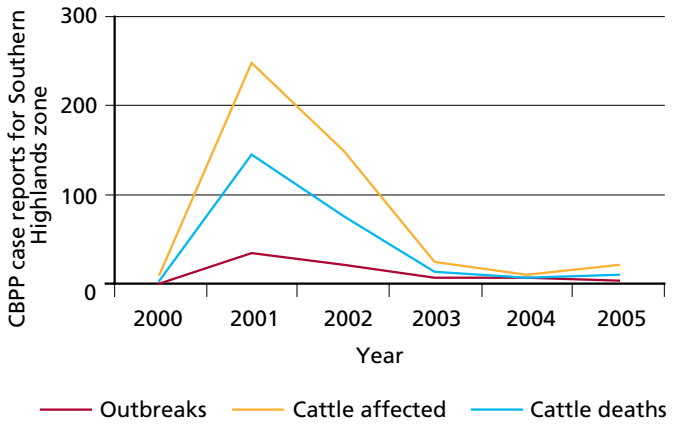
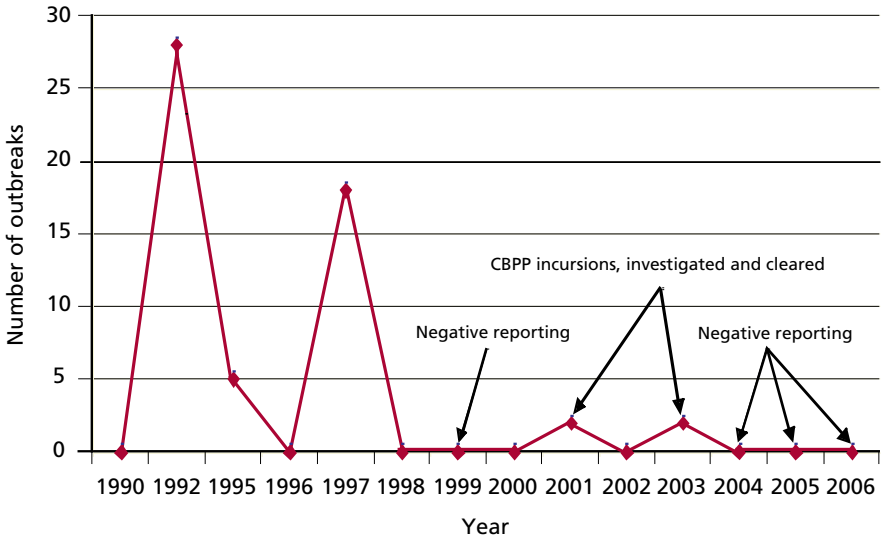


FIGURE 10.5
Number of CBPP outbreaks in Kagera



through a livestock development project (KALIDEP) that was operating in the region at that time; and (iv) communities participated fully in the vaccination exercise and in monitoring the compliance with community-based quarantines.

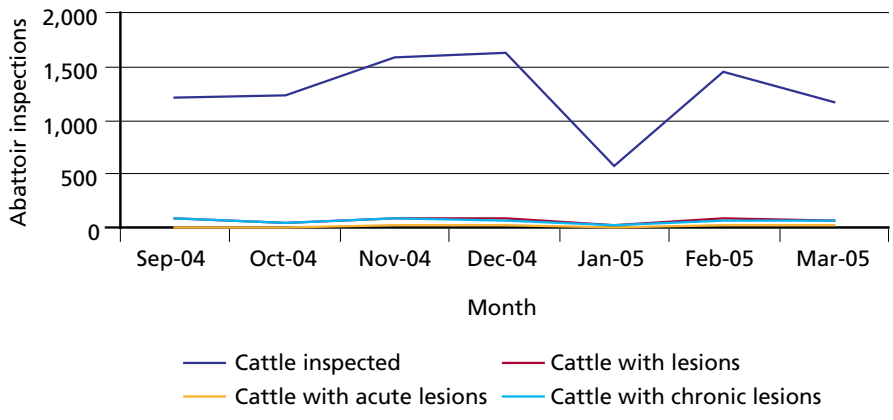
Zambia

Historical perspectives: CBPP was first introduced into Zambia (Western Province) in 1914 from Angola. It was eradicated in 1947 but reintroduced into the country in 1969 and eliminated in 1972. Zambia remained free of CBPP until 1997 when it was reintroduced into the Western Province but successfully controlled. The current CBPP was introduced again into western Zambia in 2000 by an influx of refugees from Angola. The disease has spread to all districts of the Western Province of Zambia, then to the Zambezi, Kabompo, Mufumbwe, and Mwinulunga districts in the North Western Province. The disease made an incursion into the Southern Province briefly in 2003–2004 and in 2006 positive cases was reported in the Kazungula district. In northern Zambia, CBPP was reported in the Nakonde district in 1998 and believed to be associated with the outbreaks that occurred in the same period in adjacent Rukwa and Mbeya regions in Tanzania. It then recorded cases up to 2001.

Recent–current situation: WP evidence (of endemicity), including Sesheke where epidemics have been raging. Figure 10.6 shows that CBPP is endemic in the Western Province of Zambia.

Sesheke district, Western Province: Between October 2000 and 2003 the disease had been confined to the west bank of the River Zambezi having been introduced there by an influx of Angolan refugees with about 1 000 cattle. The first case in Nawinda, on the east bank of the River Zambezi in 2003, was a result of a dowry payment from Senanga. The dowry cattle seeded the disease in Nawinda, and from there it spread along the Livingstone Road and into the district, affecting 8 of the 12 veterinary camps. Up to 2005 the Sesheke

FIGURE 10.6
CBPP reports, Western Province, Zambia



district was experiencing five to six outbreaks a month but now only one or two chronic cases are detected during inspections at slaughter facilities.

North Western Province: Since March 2004 veterinary teams have carried out six rounds of vaccination in the district. The first round vaccinated about 50 000, the second about 58 000, the third 62 000 and subsequent rounds have covered about 63 000 out of a district cattle population of 65 000, i.e. a range of 77 percent to 97 percent coverage. In the last two rounds of vaccinations, training of vaccinators, branding of cattle, community awareness and surveillance were strongly implemented components of the CBPP control in the district. CBPP incidence has dropped dramatically in the district and is confined to a few chronic cases detected in slaughter-facilities surveys. In the Mwandi veterinary camp the first CBPP case was detected in November 2003 in trade cattle from the west; vaccination was instituted and coverage was about 90 percent resulting in a rapid drop in the number of cases after the second round. The last clinical case was in May 2005 but a few chronic cases are detected on slaughter. The trend here is fairly similar to the one in the Western Province as shown by Figure 10.7.

Some success stories

Livingstone and Kazungula: As indicated above, a farmer at Bombwe who bought four animals from the Western Province introduced CBPP into the Southern Province, a CBPP-infected area. A month later two cattle died on his farm and immediately he sold the remaining two animals to a cattle trader. Of these two animals, a heifer was sold at Ngwezi in exchange for an ox and the second was sold for slaughter at Simonga slaughter slab in Livingstone (see Figure 10.8). However, because of the training and sensitization of the veterinary establishment, the veterinary assistant at Bombwe examined the lungs of the dead animals, which he had exhumed because he suspected CBPP. He then reported to the DVO who traced forward the two animals that had been sold by the farmer.

FIGURE 10.7
North Western Province abattoir findings

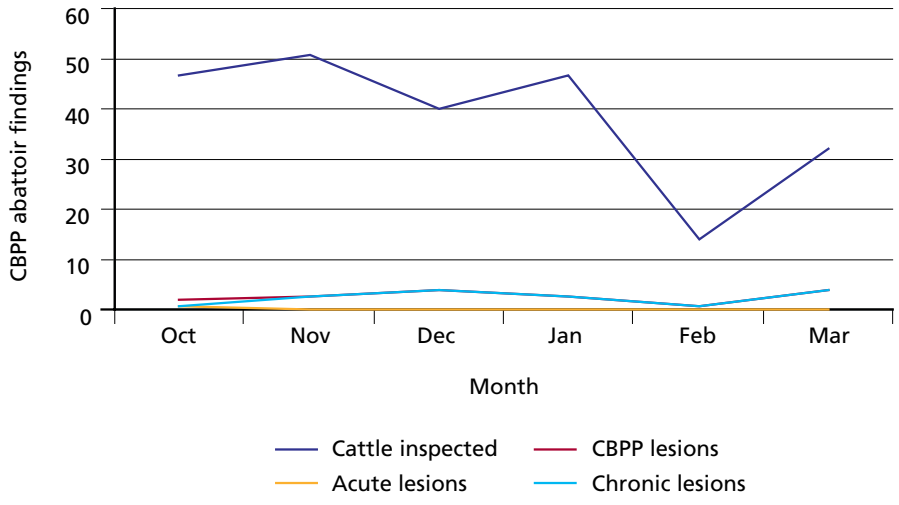
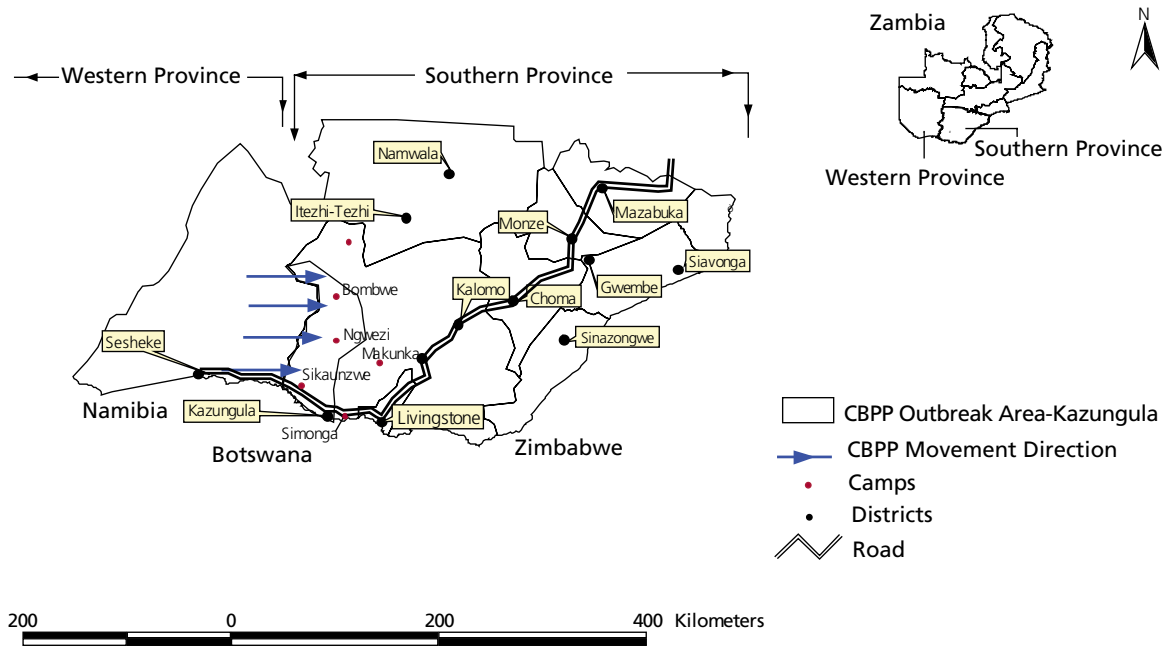


FIGURE 10.8
CBPP outbreak and advancement, Kazungula



The heifer at Ngwezi was sampled and found CFT positive but no CBPP lesions were detected upon slaughter. The one at Simonga slaughter slab was negative by serology and CBPP lesions. Both animals were slaughtered within 48 hours of discovering the disease at Bombwe.

At Ngwezi, the farmer was advised by the VO to sell all the 58 cattle on the farm for slaughter. He had to consult relatives and co-owners. A week later he agreed but subsequently changed his mind. Later, some animals started to cough and he sold the whole herd for immediate slaughter at Livingstone abattoir. The records of meat inspection show that lungs from several animals were described as abnormal. It should be noted that since there had been no prior experience of CBPP in Livingstone the DVO was not called to examine the lungs.

The DVO tested all cattle in the neighbourhood of the affected farm at Ngwezi. They were all seronegative. The testing was repeated six weeks later with the same negative results. Similarly, the remainder of the herd at Bombwe and neighbouring herds were seronegative in two repeat tests.

Sero-surveys and clinical inspections were carried out on all cattle in the surrounding districts of Kalomo, Choma, and Namwala. All animals at slaughter slabs were traced back to farms of origin. Some seropositives were detected and slaughtered. Community animal health workers found one farmer, in the Kalomo district, in August 2004 to have been harbouring illegal cattle traders and six animals were found on the farm. He was reported to the community and the DVO. The community quarantined the six animals and refused the farmer permission to graze his cattle with other stock in the village. This farmer had 22 animals. The six traded cattle were found to have brands from a CBPP-infected area

of the Western Province. The 22 animals plus the 6 traded cattle were slaughtered. Two animals were found to have CBPP lesions in the lungs and they were seropositive. In February–March 2005 a total of 625, 216, 64, 611, 340 samples were CFT tested from Choma, Kalomo, Zimba, Kazungula and Livingstone districts respectively and none showed evidence of exposure to CBPP.

Subsequently, regular clinical herd and abattoir inspections and sero-surveys have returned negative results in the district.

The success in Livingstone–Kazungula districts is attributed to: (i) sensitization workshops, public-awareness activities targeting farmers and other stakeholders; (ii) provision of pamphlets, posters and guidelines; (iii) technical assistance in strategy design; (iv) case follow-up and surveillance and (v) branding of all cattle in the district. The sensitization of stakeholders helped to set up neighbourhood watch groups – the community is now CBPP vigilant.

The DVO believes that an ongoing south African project enabled the setting-up of a tight surveillance with full stakeholder involvement and without resorting to vaccination that could have made it harder to ascertain CBPP free status of the area.

Kazungula cross-point veterinary camp: The veterinary camp is located near the cross-point to Botswana and Caprivi, Namibia and also acts as a milk-collection centre. In this area the project has assisted with branding cattle, collection of blood samples and herd inspections. In 2005 no case was detected. In 2006 there have been three seropositive cases detected.

Northern Province

Regular vaccination to create a buffer for cattle in the Northern Province in the districts of Mbala and Nakonde has been ongoing since 1999. However, available evidence is that no CBPP cases have been reported since 2001. There is indeed evidence to indicate that epidemic CBPP has not been in the area since 2005. From October 2004 through to April 2005 no CBPP lesions were detected in the 56 abattoir returns received from the Mbala district, Northern Province. Similarly, in the same period no CBPP lesions were detected in the 656 abattoir returns for the neighbouring Nakonde district in the same province. This confirms the relative disease-free status of these two districts and concurs with what has been observed in the adjacent districts of Tanzania.

CHALLENGES

It is noted that, despite apparent successes in controlling epidemic CBPP, it is not easy for the countries above to enter the OIE pathway of freedom from the disease. One of the main challenges is therefore how to manage low-prevalence CBPP, especially when the morbidities and mortalities are no longer dramatic. Generally, veterinary services (partly because of insufficient resources) tend to move their focus to more obvious and pressing issues. This allows CBPP to become truly endemic and spread to newer areas of the country that were not previously affected.

Another challenge faced by the countries in the southern Africa epidemiological cluster is the difficulty in instituting effective cattle-movement controls within countries and across international borders.

- In Angola, animal-movement control within and beyond its borders has been virtually non-existent because of civil strife that beleaguered the country for decades. In Namibia, internal movements are very much monitored and regulated but the challenge is with neighbouring countries.
- Namibia has bilateral arrangements with Angolan authorities that allow herds from either country to graze up to within 30 km of each other's country. The recent outbreaks in the Caprivi region are believed to have been because of the introduction of CBPP-affected cattle from a neighbouring country.
- In Zambia the various outbreaks (with the exception of that in the Northern Province) mentioned above have all been attributed to infected cattle from Angola. Within Zambia, the spread from the Western Province to the North-western Province and to the eastern bank of River Zambezi as well as to the Southern Province has stemmed from factors that compromised animal-movement controls.
- In Tanzania, the most challenging cattle movement is that by migrating pastoralists, and the Veterinary Services expects to tackle this in collaboration with the recently established Directorates of Pastoral & Rangeland Development and the Animal Identification & Traceability. In the long term, there will be a need to harmonize animal identification regionally.

To effectively enter and work through the OIE pathway, the key factor is provision of science-based evidence to support the claim of freedom from the infection or disease. Essentially, this requires both active and passive surveillance; countries are experiencing difficulties here because of inadequate funding of the veterinary services. Recently, Tanzania designed a surveillance strategy for the Southern Highlands but its sustainability hangs in the balance because of inadequate resources. Inadequate resources also inhibit countries from progressing to the "test and slaughter" policy when the epidemic CBPP has been overcome.

Consolidation of gains made so far and completing the work already started in the current climate of threat of highly pathogenic avian influenza (HPAI) and other exotic emerging disease could be compromised through relegation of CBPP to low priority because it is not so dramatic in the low prevalence state.

Another challenge is the assessment of the socio-economic impacts of the outbreaks on the victims of the outbreaks, bearing in mind the losses but also the impact of instituted control measures. This is extremely important as it will direct the mitigation measures for the victims, determine the cooperation from the cattle owners and their participation in the community (surveillance) policing that is very much critical to disease-intelligence systems. This assessment will also provide a platform for justification of resource allocation and better preparedness by governments.

CONCLUSIONS AND POSSIBLE WAY FORWARD

The emergency phase, which the CVOs recommend in 2003, has indeed brought CBPP epidemics under reasonable control in Namibia, Tanzania and Zambia. Now the next stage is to move into the recovery stage where CBPP at low prevalence is eliminated through intensive surveillance that would lead into "test and slaughter". The situation in Angola is not yet clear but needs proper investigations and the current attempts to establish an infrastructure for investigating CBPP and other livestock diseases in the country are welcome.

The above indicates that CBPP epidemics can be controlled in the region and even eradicated in some cases. However, this will require the countries to scrutinize the success stories and analyse what enabled success to be achieved in those circumstances to come up with lessons learnt in order to build up on those experiences for future successful progressive CBPP control. This will require actual field operating personnel like the DVOs, together with epidemiologists and laboratory support staff from the countries within the epidemiological clusters mentioned in the introduction, to share the experiences from their countries and carry out analysis of why successes were achieved in certain situations and failures experienced in others. Based on the analysis, realistic plans for controlling and eventually eradicating CBPP from their localities could then be prepared. This should feed into the overall national control and eradication strategy, which should be funded within the available resources.

Presentation 11

The current situation of CBPP in Zambia

Prospects for control

J.M. Belemu,[1] M.P.C Mangani,[2] F.L. Musisi,[3] S.H. Kabilika,[4] L. Munsimbwe[4]

INTRODUCTION

The agricultural sector in Zambia has three main categories of farmers as shown in Table 11.1. Of the country's total area of 753 000 square kilometers, almost one-quarter (9 million ha) is arable land, but only 16 percent of this is cultivated annually. Agriculture provides a livelihood to about 50 percent of the population and 67 percent of the economically active. It also remains by far the main opportunity for income and employment for women who comprise 65 percent of the rural population.

The situation of major transboundary animal diseases in Zambia (2001–2005) is summarized in Table 11.2. The emergency control of epidemic CBPP in Zambia is an effort fitting into the regional concept of controlling and eradicating transboundary disease (TADs) from southern Africa. The project activities were specific to the attainment of containment of CBPP in Zambia and to creating a sustainable base for control and eradication of the disease. These activities are based on the SADC implementation framework drawn by Directors of Veterinary Services in the region in 2003.

The implementation of selected activities was centred on achieving freedom from the disease in affected areas while preventing entry or re-entry in free and controlled areas respectively. The project set a base that has allowed the Zambian Veterinary Service to conform with the principles for assessing the disease status (four-stage pathway) as developed by the OIE through provision of CBPP vaccines, implementing quality vaccination programmes, institutionalizing surveillance, logistical support – transport, training field staff – and overall coordination of the control strategy.

ACHIEVEMENTS

- Currently, the CBPP situation in the Southern Province is clearly known owing to consistent surveillance activities. Despite the disease having broken out in early 2004 in Bombwe and Ngwezi, it has not established itself in these areas. This is very significant for attaining freedom from the disease. There is evidence that the herds in

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[2] Director, Veterinary Services, Dept., Zambia
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[4] Veterinary Services, Dept., Zambia

TABLE 11.1
Categories of farmer, Zambia

Category	Total	Area cultivated (ha)	Inputs	Labour
Small scale (traditional)	600,000	1–2	Hand hoe	Family
Emergent	50,000	5–20	Animal draught power	Family, hired
Large scale (commercial)	740	> 40	Machinery	Hired

TABLE 11.2
TADs incidences

Disease	Disease Incidences – Countrywide									
	2001		2002		2003		2004		2005	
	Cases	Incdc (%)	Cases	Incdc (%)	Cases	Incdc (%)	Cases	Incdc (%)	Cases	Incdc (%)
FMD	182	0.007	221	0.009	–	–	450	0.02	–	
CBPP	–	–	13,462	0.54	7,434	0.31	543	0.023	125	0.005
ASF	497	0.02	188	0.007	265	0.01	44	0.002	–	
LSD	1,599	0.065	735	0.03	429	0.02	517	0.02	694	0.03
ND	6,747	0.27	8,050	0.32	4,700	0.2	9,477	0.4	4,447	0.2
RVF	There are no cases that have been recorded in Zambia									

these areas were actually in contact with Western Province cattle and possibly could have been incubating the disease, but due to effective measures the disease has been eradicated. The strategy of testing and slaughtering of suspected or infected cattle at the abattoir brought the disease under control quickly. This strategy was applied systematically and with close supervision so that affected households were assisted in receiving payment for their slaughtered cattle at commercial rates. This has assisted in the recovery of these households.

- In order to make the control attained so far sustainable, the project embarked on public-awareness programmes to sensitize the public on the disease and its control measures. To curb illegal movements, cattle identification by branding has been carried out in Sesheke and Kazungula to identify cattle in these areas.
- The DVLD is carrying out disease-control programmes as an ownership process. Currently, the veterinary service is able to assess the achievements of interventions being carried out in the field effectively, particularly CBPP control, while previously it was difficult to verify and evaluate the CBPP control programme.

LIVESTOCK-MOVEMENT PATTERNS

Livestock movements are generally from the main traditional cattle- rearing areas of the Southern Province and Western Province. Because of CBPP in the Western Province, no live animals are allowed out of the province. Animals are slaughtered at abattoirs and slaughter slabs within the province and transported in refrigerated trucks to Lusaka and Copperbelt.

The Southern Province has live animals moving out of the province for slaughter and breeding. These animals move after issuance of a livestock movement permit. Staff veterinarians at veterinary checkpoints at Kafue Bridge verify that all animals coming from the Southern Province have the necessary documentation. The department has three road checkpoints, which are strategically located on the main highways leading into the Central and Lusaka provinces from the three major cattle-raising regions of the country – Southern, Western and Eastern Provinces.

CURRENT SITUATION

The economic importance of containment and eventual eradication of CBPP regionally cannot be overemphasized. In 1996, Botswana to the south of Zambia spent up to 400 million Pula (approximately US$100 million at the time) to eradicate CBPP incursion in that country; it is still contending with compensation following adoption of CBPP eradication by a slaughter policy. Namibia – another neighbouring country to the south – has restricted CBPP along the northern strip that borders Angola and Zambia by intensive vaccination of more than half a million cattle annually. Zimbabwe (another country to the south of Zambia and with direct rail and road links at the famous Victoria Falls) has been free of CBPP since the start of the last century.

All three countries, Botswana, Namibia and Zimbabwe have until recently been enjoying good returns on livestock product exports to lucrative markets like the European Union (EU). Many of the cattle that form part of this trade originate from small-scale livestock producers passing through feedlots and bigger commercial livestock farms into the approved abattoirs for beef export to European markets. Therefore, the current CBPP situation in Zambia is not only a national problem but has direct implications for the countries mentioned south of Zambia. If the disease were to cross into those countries, it would also threaten South Africa, Swaziland and Lesotho, which have all been CBPP free for more than 70 years. The threat of CBPP spread from the Zambian Northern Province has also potential serious implications for Malawi and Mozambique which are also currently CBPP free. It must be noted that veterinary services of these two countries have severe financial and human capacity limitations and entry of CBPP would easily gain a foothold there and pose another serious threat to the rest of the CBPP-free southern African countries.

CBPP SPREAD

The current CBPP crisis in Zambia is understood to be due to interference with the original operations of the CBPP cordon line and lack of financial resources to implement sustained effective active CBPP surveillance, which should be accompanied with appropriate and practical control/eradication measures. The situation was exacerbated by the recent unprecedented floods of the Zambezi river following record-breaking rainfall, noted in some areas such as Kalabo to have been 1 480 mm compared to the average of 906 mm. The outcome was that most communities in the plains were forced to move their livestock to higher grounds, which put pressure on the grazing land, thus further reducing coping mechanisms for livestock rearing in the area. In addition, this caused the spread of CBPP to previously disease-free areas following mixing and crowding of cattle populations in

the higher areas. The movements on the western side of the Zambezi have led to CBPP outbreaks as far as the Caprivi Strip of Namibia while on the eastern side of the river this contributed to the disease spreading out into parts of Shesheke district, which were until then disease free.

The high morbidity and mortality of cattle particularly in new CBPP-affected areas, such as Chavuma/Zambezi and Shesheke, compelled households to sell their cattle; in the case of Shesheke, this led to infected cattle crossing into the Southern Province of Zambia. At the same time, floods reduced livestock market value in remote areas because access to markets became difficult. The net effect was further reduction in availability of the coping mechanisms of the affected communities; they could not easily acquire grain and other necessities. The floods also disrupted the CBPP vaccinations programmes as most areas became inaccessible.

Whenever CBPP is detected in an area, quarantine is imposed and also a ban on animal movement, affecting cattle keepers and small-scale farmers severely. Cattle rearing used to be a major livelihood but, since CBPP outbreaks, cattle populations have greatly diminished, thus threatening livelihoods dependent on it. As a livelihood, cattle production provides financial means to meet various needs and thus serves as a bank on hooves, draught power and manure to cattle and non-cattle-owning households. The latter aspects are realized through cattle hiring/renting schemes. The manure aspect is particularly important because the soils in the CBPP-affected areas are poor so manure not only serves to fertilize the soil but also to improve its texture and its moisture-holding capacity leading to better crop production. The draught power dimension is very important in the cultivation and transportation in rural areas affected by this disease; the acreage covered by oxen ploughing versus hand-hoe cultivation is far larger. The current CBPP epidemic in the affected areas further exacerbates the already difficult situation in which HIV/AIDS pandemic is devastating communities. These communities cannot afford the stressful ordinary hoe cultivation and haulage of various loads within their rural environments.

Livestock production, and in particular cattle rearing, is a very important aspect of the livelihoods of rural populations in the CBPP-affected and at-risk areas of Zambia and thus central to food and livelihood security assurance. Addressing the CBPP outbreak constraint will greatly reduce dependence of these people on food aid and set them on sustainable livelihoods.

CONTROL PROGRAMMES

The current strategy of controlling CBPP in Zambia is geared towards vaccinations. However, the policy of the Zambian government is to control and eradicate CBPP once introduced in Zambia or spread to new areas.

Presentation 12

Control of CBPP in Tanzania

Current status and the implementation of a roll-back vaccination plan

P.Z. Njau[1] and J.I. Kitalyi[1]

INTRODUCTION

The economy of Tanzania heavily depends on agriculture (crops, livestock, fisheries and forestry). The livestock sector contributes an average of 30 percent of the agricultural gross domestic product (GDP), of which 40 percent is from beef, 30 percent milk while poultry and small stock is about 30 percent. Agricultural households rely heavily on livestock as a major source of livelihood with zebu cattle accounting for 50 percent and being the dominant livestock species (see Figure 12.1). Livestock play various roles in livestock-keeping communities but in particular they provide relatively secure forms of incomes, savings, and investment and as a buffer against crop failure.

The country has an estimated 18.5 million heads of cattle (of which 98 percent are found in the traditional sector), 12.5 million goats, 3.6 million sheep, 1.2 million pigs and about 33 million rural chickens. With this enormous livestock resource, the country has a significant potential to secure export markets for livestock and export products.

Most of the meat produced in the country comes from the traditional sector dominated by the Tanzania shorthorn zebu (TSZ). It is estimated that about 1 500 000 cattle, 2 500 000 goats and about 550 000 sheep are slaughtered annually countrywide, giving an average production of about 335 000 tonnes of meat annually. This is used for local consumption but some is now being exported. The main reason for not being able to capture big export markets is the presence of transboundary diseases such as contagious bovine pleuropneumonia (CBPP) and FMD.

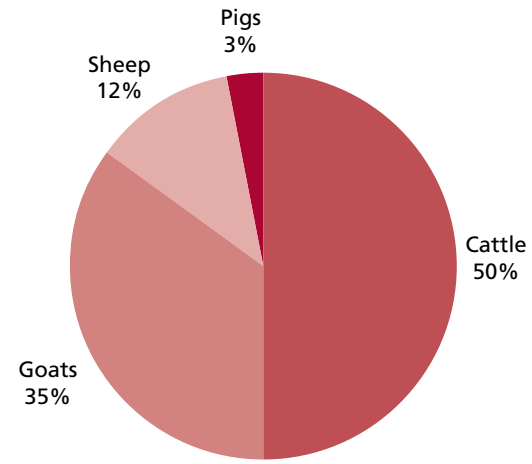
The first outbreak of CBPP was reported in 1916; this occurred in Loliondo on the northern border neighbouring Kenya. Control measures which included strict animal movement control and vaccination using Kabete broth vaccine brought the disease under control by 1946.

There was a second outbreak in the Loliondo division at Nguserosambu in 1955. The same Kabete broth vaccine accompanied by strict animal-movement control measures managed to contain the disease by 1964.

The third and current raging outbreak occurred in 1990 at Soit Sambu, again in the Loliondo division after an absence of the disease for more than 25 years. Purchase of bulls

[1] Department of Veterinary Services, Ministry of Livestock Development, Dar-es-Salam, Tanzania

FIGURE 12.1
Head of livestock by type



Source: Ministry of Ariculture (ASDP 200

from the neighbouring country for breeding purposes is believed to be the source of the disease. The disease was spread to nearby districts through rustling of cattle. However, in 1992 a new focus of the disease unrelated to the one in Loliondo occurred in Kagera region and it was established to have originated from Rakai in Uganda. In 1994 the disease spread south of the Central Railway Line and since then to various parts of the country. When CBPP outbreaks occur in areas without recent experience of the disease, it results in the death of many cattle. CBPP can be introduced into a new area by a few subclinically infected cattle.

In areas where the disease has become endemic, the cattle death rates are not dramatic but the chronic coughing and respiratory distress make such animals unthrifty and therefore they fetch poor prices in the livestock markets.

CBPP CONTROL EFFORTS

The role of the government in the combating of CBPP has centred on defining priorities and formulating national control policy, implementing a strategy and monitoring and evaluating the control programme. The control of the disease has mainly been through restricted animal movements and mass vaccination campaigns. In the control of livestock movement, quarantine has been imposed in the affected areas to prevent animals moving out or into the area. However, enforcing these quarantines has been difficult because of the country's vastness, inadequate resources and the lack of livestock farmers' compliance.

Livestock movement control has been a major challenge in the control of the disease. The main reasons for livestock movement are trade stock moving to markets, translocation of stock to new areas and pastoralists' movement in search of water and pastures. In recent years, because of intermittent drought, there have been large cattle movements from the north to southern parts of the country. In 2002 a Presidential Circular came into

force which prohibited long-distance trekking of trade stock in favour of trucking and the use of railway wagons.

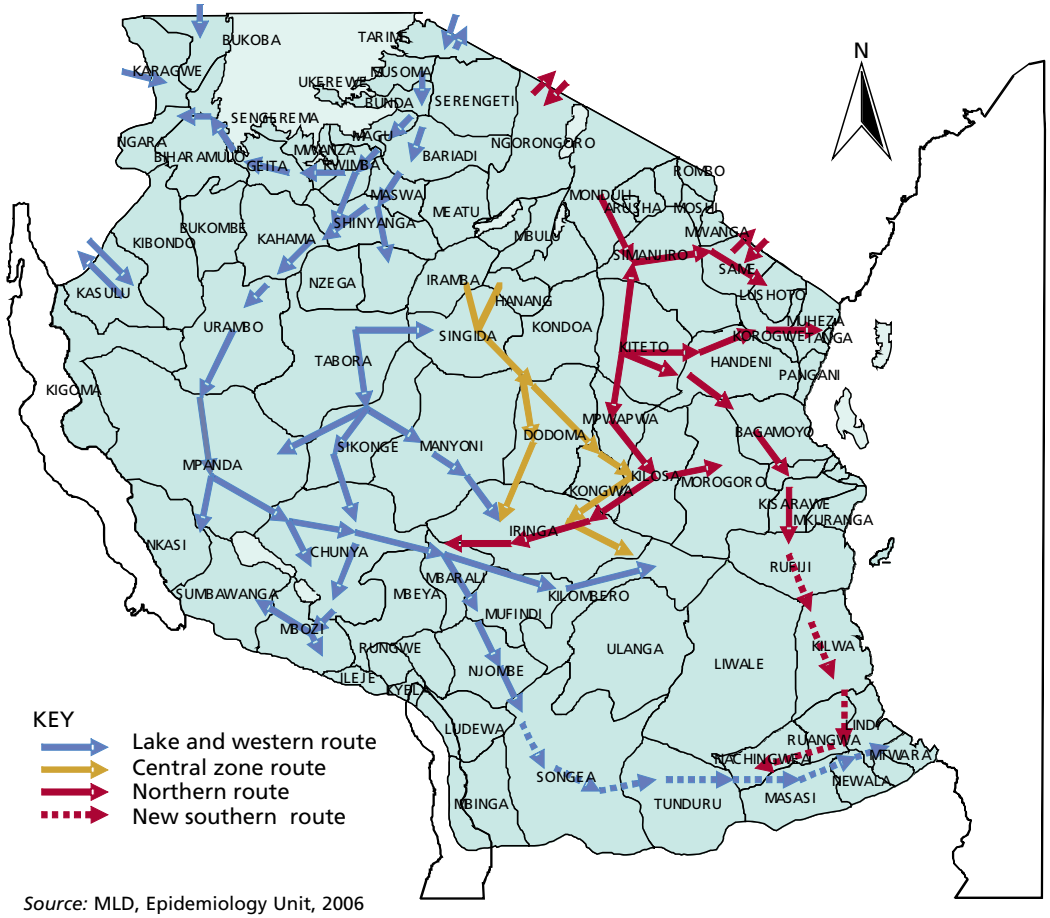
In order to control livestock movement there is an established zoosanitary network consisting of 40 border-posts, 381 internal checkpoints, 20 quarantine stations and 19 holding grounds. These facilities need enormous resources to rehabilitate and become operational.

The Presidential Circular has now been re-enforced by the enactment of the Animal Diseases Act Number 17 of 2003. This has instituted compliance with the legislation, and it is now mandatory to transport trade animals by trucks, train wagons and ferries; this has arrested further spread of CBPP.

A significant challenge to livestock movement is posed by migrating pastoralists (see Figure 12.2), but this is being tackled by serious government efforts to resettle them in new areas which have been identified and which have adequate pastures and water. In this new initiative the government will eventually provide land rights for the pastoralists who will be required to keep animals according to the carrying capacity of the land allocated to them.

Implementation of the programme for control of CBPP by vaccination started in 2000 with the aim of conducting a baseline survey, annual vaccinations campaigns, post-vac-

FIGURE 12.2
Pastoral livestock movement trends, Tanzania



Source: MLD, Epidemiology Unit, 2006

cination surveillance and monitoring and economic impact assessment. The target was to vaccinate all cattle in the disease-risk areas with the ultimate goal of preparing the country to embark on the OIE pathway for CBPP elimination as indicated below in Figure 12.3.

THE ROLL-BACK PLAN

Strategy

The control strategy for the disease in the country is still based on mass vaccinations and movement control coupled with strong surveillance and diagnostic backup. The MWLD economic impact assessment of the CBPP vaccination study shows that the numbers of animal vaccinated range from 1 623 649 in 2000 to 5 706 807 in 2004. These figures show that less than one-half of the 9 000 000 cattle estimated to be in infected and in high-risk areas were initially vaccinated. The study reveals that response from vaccination campaigns was very low initially (about 24 percent in 2000) and improved gradually through 2005 (55 percent in 2004; 60 percent in 2005). The maximum vaccination coverage reached is within the range of the projected national figures of 60–70 percent. The low response is partly attributed to the fact that some livestock keepers were reluctant to participate fully due to vaccination reactions and the introduction of cost recovery in the initial stages.

Rationale

Amongst the major transboundary animal diseases present in the country, contagious bovine pleuropneumonia (CBPP) is currently the most important economically. The roll-back vaccination plan aims at systematically concentrating resources and efforts in specific zones with the aim of eliminating the disease zonally, starting from the south where there are still many clean areas. Implementation of the plan started in 2003 with the Southern Highlands Zone bordering Zambia and Malawi. With the assistance of FAO it was possible to implement the roll-back plan through supporting government efforts in the interventions planned. For the purpose of implementing the roll-back plan the country was subdivided into the zones shown in Figure 12.4.

As there have been few incidences of the disease in Kagera region following intensive vaccinations, these areas have been designated as "surveillance zones" for monitoring

FIGURE 12.3
CBPP elimination – OIE pathway procedures

No clinical disease detected for two years after vaccination or last case.

Declaration of Provisional freedom from the CBPP within the country.

Effective measures are in place to prevent re-introduction of the disease.

Country declare free from CBPP.

No vaccination done against CBPP for ten years.

Country declare free from CBPP infection.

Obtain certificate of freedom from CBPP infection by OIE.

disease outbreaks in the infected zones. Surveillance will also be carried out in areas where zero grazing is being practised.

In the roll-back vaccinations, cattle in each implementing zone will be vaccinated three times in the first year followed by annual vaccinations for at least four years after which the zone will graduate to a surveillance zone.

CBPP TRENDS

In order to establish the trend of the disease, a number of activities have been conducted including passive and active surveillance. Some of the results are as shown in Table 12.1, and Figures 12.5 and 12.6. Trends indicate that the disease has been decreasing since we started implementing the rollback plan in 2003 (Table 12.2).

FIGURE 12.4
Zones for implementing the roll-back plan - Tanzania

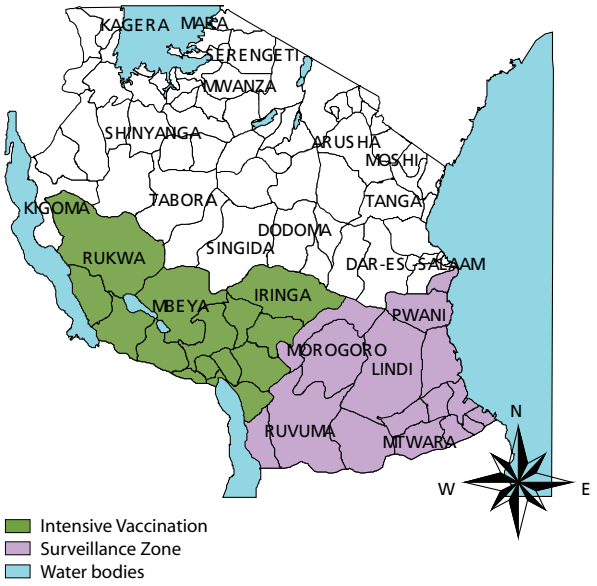


TABLE 12.1
Abattoir CBPP cases in Southern Highlands Zone, January 2003–June 2005

Period	No. of animal slaughtered	CBPP cases found
January–June 2003	33,178	92
July–December 2003	29,688	66
January–June 2004	24,910	30
July–December 2004	31,656	20
January–June 2005	31,468	13

Source: MLD reports 2006.

FIGURE 12.5
Animals slaughtered in abatoirs in Southern Highlands

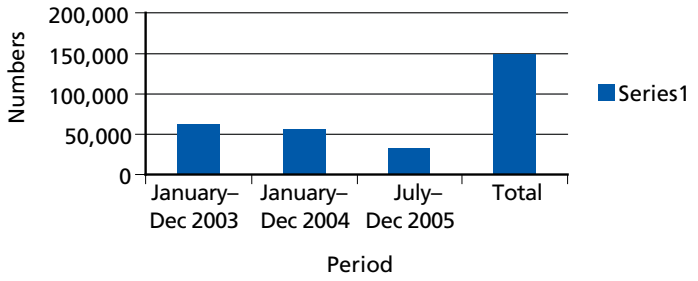


FIGURE 12.6
6 CBPP cases found in abattoirs in Southern Highlands

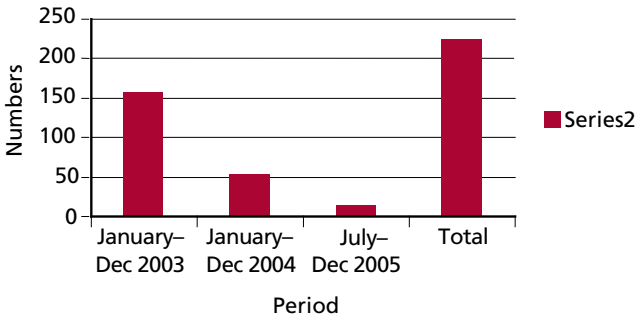


TABLE 12.2
CBPP cases, reports, 1998–2005

Year	Districts reporting CBPP	No. of outbreaks	No. of cattle affected	Deaths
1998	32	39	4894	3217
1999	35	216	8014	3445
2000	44	139	7,632	4,821
2001	29	83	4,302	3,442
2002	28	72	3,911	1,533
2003	26	82	2,669	1,149
2004	17	27	2,126	716
2005	16	34	573	223

Source: Epidemiology unit. MLD, Tanzania

Success of the roll-back plan

When work started in 2003 the baseline abattoir surveillance indicates that 6 out of the 13 districts involved reported the disease; by 2005 11 out of the 13 districts did not report the disease. In the two reporting districts cases had gone down dramatically from 133 in 2003 to 6 in 2005.

Lessons from roll-back plan

Spread of disease has been arrested after movement of animals was contained through the Presidential Circular which clearly demonstrates its role in preventing the spread of the disease. The cooperation of livestock keepers can be achieved when they are aware of the disease and are willing to participate in control measures being undertaken. Without their cooperation it is difficult to reach coverage targets anticipated.

THE WAY FORWARD

In order to succeed in CBPP control it is important to do the following:

- Allocate resources to continue with the roll-back plan.
- Strengthen laboratory capacity to assist surveillance and confirmation of the reported cases.
- Increase livestock farmer participation through participatory disease-search training and compliance to legislation.
- Introduce modern diagnostic methods and validation of diagnostic tests.
- Strengthen the newly established unit of Animal Identification and Traceability within the Ministry of Livestock Development.

Presentation13

Africa Livestock (ALive) CBPP Research Programme

F. Thiaucourt [1]

INTRODUCTION

The Forum for Agricultural Research in Africa (FARA), the African Union Inter-African Bureau for Animal Resources (AU-IBAR) and the International Livestock Research Institute (ILRI) are leading the development of the research component of the African Livestock (ALive) Programme with full stakeholder participation and ownership. The broad objective is to address priority opportunities and constraints facing the livestock sector in terms of the potential for improving the livelihoods of the poor, determining the pathways out of poverty and conceiving, developing and validating technical, policy and marketing innovations that will result in sustainable development. The ALive initiative was first sponsored as a concept by the World Bank in 2001 and has since benefited from input from many African livestock stakeholders. It was officially launched in June 2004. FARA, AU-IBAR and ILRI have facilitated the development of research proposals aimed at addressing constraints on sustainable access to markets for livestock and livestock products, particularly those related to animal health, including strengthening of livestock services and institutions.

AU-IBAR has identified CBPP as the second most important animal health constraint on African livestock production and, therefore, supply of beef cattle for marketing. It is endemic in over 26 Sub-Saharan African countries and is unlikely to be eradicated in the near future. Present control measures are constrained by the inadequacy of the available diagnostics and less than optimal targeting of the available vaccines, while curative measures using antibiotics have had limited success. Advances in science have opened up opportunities for developing new vaccines that could provide longer immunity and differentiation of vaccinated from infected individuals, which is required to make the eradication of CBPP a pragmatic possibility.

This research proposal is aimed at developing improved and more efficient control of CBPP through integrated use of diagnostics, vaccines and treatments. This project is compliant with the guidelines and criteria of the Framework for African Agricultural Productivity (FAAP) and consistent with the goals and objectives of the Comprehensive African Agricultural Development Programme (CAADP) of New Partnership for Africa's Development (NEPAD).

OBJECTIVES

The objective is to develop an improved and more efficient system of controlling CBPP through an optimal use of appropriate existing and new diagnostics, vaccines and treatment. This will be achieved through the following specific objectives:

[1] CIRAD-EMVT, Montpellier, France

1. Development of decision-support tools based on cost–benefit analysis using disease prevalence and economic-impact assessment.
2. Development of technological tools, namely vaccines that provide longer protection, are thermostable and compatible with the differentiation of vaccinated and infected animals (DIVA concept), diagnostic tests that allow evaluation of vaccination campaigns, early detection of infected animals and chronic carriers and evaluation of the effect of treatment on CBPP transmission.
3. Enhancement of the capacity of African veterinary services and other stakeholders for collaboration in improved disease control.

ACTIVITIES

Development of decision-support tools: The prevalence and economic impact of CBPP on individual countries and the regions will be determined through enhanced disease-surveillance systems conducted through various CBPP control activities. Spatial epidemiology and dynamic mapping of animal densities and movements will be conducted to determine the prevalence and incidence of CBPP. This may be facilitated by access to sera collected for PARC purposes. In order to understand CBPP transmission dynamics, mathematical models will be developed for different cattle husbandry and ecological systems.

Development of technological tools: Gene targeting and the evaluation of attenuated strains will be studies for the development of DIVA vaccines. Adjuvants that trigger significant and longer protection induced by inactivated vaccines will be evaluated. Thermostable recombinant vaccines will be developed and evaluated. Companion diagnostic tests will be co-developed that should be specific, sensitive, user friendly, robust, cost-effective, validated under OIE guidelines and produced under good manufacturing practices (GMP). They should be able to detect acutely and chronically diseased animals. The efficacy of antibiotic therapy and combination therapy with antibiotic and anti-inflammatory drugs will be investigated.

Capacity building: The capacity of African veterinary personnel and diagnostic laboratories will be enhanced through training in the use of the new methods and tools related to CBPP indicated above.

OUTPUTS AND IMPACTS

The project will provide five outputs:

1 Decision-support tools for determining the prevalence of CBPP and assessing the economic impact of CBPP based on spatial distribution, mapping of animal population densities, tracking and movements and validated models of CBPP dynamics.
2 Diagnostics that allow evaluation of vaccination campaigns, early detection of infected animals and detection of chronic carriers and improved treatment of CBPP.
3 Thermostable DIVA vaccines that provide longer protection.
4 Improved control strategies for CBPP based on integrating use of vaccine, diagnostics and treatment.
5 Enhanced capacity of African veterinary services and other stakeholders to collaborate in improved disease control.

Presentation 14

CBPP research in Portugal

New outlooks in post-eradication era

A. Botelho [1]

OCCURRENCE OF CBPP IN PORTUGAL

In Portugal CBPP reappeared in 1983 after 30 years of supposed absence. The north-west regions were most affected (Entre Douro, Minho, and Beira Litoral). In 1985 the Official Veterinary Services implemented an eradication program, revised in 1989 with EU funds and updated each year. The last outbreak of CBPP in Portugal occurred in 1999 in the region with the highest CBPP prevalence – Entre Douro e Minho.

CBPP CONTROL AND ERADICATION IN PORTUGAL

The main aspects that contribute to controlling and eradicating CBPP were:

- Geographical limitation and definition of three main regions, according to the prevalence of the disease: infected region with high prevalence (including: outbreaks area and surveillance area); buffer region and free region.
- Diagnostic tests (CFT) – to bovines from all herds of the infected region, twice a year or once a year, depending if they were from outbreak region or surveillance region; CFT to 50 percent of bovines from the buffer region, once a year; CFT to 10 percent of bovines from the free region, once a year.
- Quarantine of herds with positive CFT, slaughter of CFT-positive animals and CBPP confirmation by bacteriological diagnostic and polymerase chain reaction (CR).
- Stamping out in those herds where *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC) was isolated.

Since 1998, immunoblotting (IBT) was introduced as a routine, confirmatory serological test and was successful in reducing the number of slaughtered animals. From 20 588 IBT tests, performed during 1998 to 2002 on CFT positive or doubtful sera, only 64 corresponded to a specific profile. In 2003, Portugal was considered free from CBPP without vaccination by the International Committee of the OIE. Among others, the main obstacles to the control and eradication of CBPP are the sensitivity of serological diagnostic tests, the absence of effective vaccines and uncontrolled cattle movement.

CBPP SURVEILLANCE IN PORTUGAL

Since 2001, 10 percent of bovine herds are screened for CBPP once a year by CFT, performed in 12 regional laboratories, and positive or doubtful results are confirmed by IBT in

[1] Laboratório Nacional de Investigação Veterinária, Lisbon, Portugal

the NRL–LNIV. During the last five years (2001–2006) 17 985 IBT tests have been performed in the frame of the Portuguese surveillance plan for CBPP.

MILESTONES IN CBPP RESEARCH, CONTROL AND ERADICATION IN PORTUGAL

During 18 years of research in CBPP, contributions have been made to: improvement of CFT as the official screening test for CBPP (1984–1988 – Regalla *et al.*); routine use of IBT as a CBPP confirmatory serological test, approved in 2004 by OIE (1991–1998 – Gonçalves *et al.*); evaluation and implementation of PCR systems, for specific detection and identification of *Mmm*SC, and development of molecular typing methods (1992–1996 – Botelho *et al.*); molecular characterization, detection and typing of *Mmm*SC (1996–2002 – Botelho *et al.*).

In these studies epidemiological markers were developed (IS*1296* RFLP) to trace sources and routes of infection of CBPP. A new and valuable tool for molecular taxonomy, based on transfer RNA (tRNA) profiles, was able to differentiate between members of the *Mycoplasma mycoides* cluster. In addition, the differentiation between European and African strains by PCR and IBT was implemented, allied to the differential serological detection of infected cattle by IBT using LppB-His (38 kDa) specific antigen.

NEW OUTLOOKS IN THE POST-ERADICATION ERA

In the post-eradication era we are willing to contribute with our experience for the control of CBPP in African countries, collaborating in those more pertinent aspects like application of more effective diagnostic tests and vaccines. Portugal can provide training and cooperate to implement sensitive, specific and rapid field diagnostic tests for early detection of CBPP. We are looking forward to being able to test our routine IBT diagnostic with bovine sera from CBPP outbreaks in Africa and this can only be done in the framework of FAO-OIE and IAEA.

Annexes

Closing remarks

JUAN LUBROTH, ANIMAL HEALTH SERVICE, AGAH

Ladies and Gentlemen,

Have we made some progress from the 3rd Consultative Group Meeting to this one, the 4th? Are we making a big leap forward?

In my opinion we have and we are. In consideration of Chapter 2.1.1 of the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals on CBPP*, it rests on us to do the work to answer some as yet unanswered questions that may eventually affect the contents of this chapter and especially in the control options that may become available to us. It is up to us to work on the performance indicators of the success of antibiotic therapy.

Thank you.

SAMUEL JUTZI, DIRECTOR, ANIMAL PRODUCTION AND HEALTH DIVISION

Ladies and Gentlemen,

You are an assembly of the world's top experts on CBPP. I am honoured that you have taken the time to participate in the 4th Consultative Group Meeting on CBPP.

The recommendations that I have heard today reflect consensus after deep and prolonged deliberations that considered the pros and cons of the subject of antibiotic use and vaccination. In particular, the decisions on antibiotic use were taken after deep analysis and real world considerations. Progress has been made that has taken courage collectively as a group and this has been done without shirking responsibility and hiding behind the need for more research – congratulations!

Thank you for spending time at FAO, Rome and I hope that you agree that the time was well spent.

Thank you.

WILLIAM AMANFU, ANIMAL HEALTH SERVICE, AGAH

Ladies and Gentlemen,

I would like to thank the Chief of Animal Health Service and the Director of Animal Production and Health of FAO, for their kind words. Thank you for finding the time away from the heavy pressures of HPAI and other aspects of work at AGA.

This meeting would not be possible without the participants and we hope that you understand the pressures at FAO. In this meeting we took on a very controversial aspect of CBPP control. I thank the working group leaders for taking the discussions forward and to the point that we have all reached. I would like to thank the translators and Lucy Mensah (Secretary) for all their efforts.

Thank You.

[The Group thanked Willie Amanfu for his efforts in hosting this meeting.]

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Agenda

Secretariat: FAO Animal Health Service, EMPRES/Infectious Diseases Group
Rapporteur: John Bashiruddin, IAH-UK
Theme: Control of CBPP: Antibiotics to the rescue?

MONDAY, 6 NOVEMBER 2006 – PHILIPPINES ROOM, C 277/281

Session Chair		**Prof. V. Caporale, IZS, Teramo, Italy (OIE)**
09:00 – 09:15	Welcome Address	Dr Joseph Domenech CVO/Chief, Animal Health Service-FAO
09:15 – 09:30	Opening Remarks	Dr Samuel Jutzi Director, AP & HD-FAO
09:15 – 09:25	Principal objectives of the Consultation and expected outputs	J. Lubroth, FAO
09:25 – 09:50	The use of antibiotics for CBPP control: The challenges	W. Amanfu, FAO
09:50 – 10:20	Break for Coffee/Group Photograph	

CBPP Control: Antimicrobial Research

Session Chair		**Dr J. Domenech CVO/Chief, Animal Health Service-FAO**
10:20 – 10:40	Effect of antibiotic therapy on the pathogenesis of CBPP: Experimental transmission of the disease by contact from infected animals treated with oxytetracycline	Dr M. Niang, CVL Bamako, Mali
10:40 – 11:00	Experimental and field use of danafloxacin to control CBPP	R. Nicholas, VLA, UK
11:00 – 11:20	Mathematical modelling of CBPP transmission: control methods and the potential impact of antibiotics	J. Mariner, ILRI, Nairobi-Kenya
11:20 – 11:40	Comparative studies on the *in vitro* antimicrobial sensitivities of *MmmSC* and *M. bovis*	R. Ayling, VLA, UK

11:40 – 12:45	Discussions on antimicrobial research on CBPP	
12:45 – 14:20	Lunch break	

CBPP Vaccines/Molecular Research

Session Chair		**J. Lubroth Senior Officer EMPRES/IDG**
14:20 – 14:40	Multilocus typing for a finer differentiation of *Mmm*SC strains	F. Thiaucourt, CIRAD/EMVT Montpellier, France
14:40 – 15:00	Evaluation of the xerovac process in the preparation of heat-tolerant CBPP vaccine	J.K. Litamoi, FAO/AU-IBAR Nairobi, Kenya
15:00 – 15:20	Discussions	
15:20 – 15:40	Break	
15:40 – 16:00	Development and validation of new technologies for control of CBPP in east Africa	C. Schneir Moredun Institute Edinburgh, UK
16:00 – 16:20	The Effect of Cyclosporin A (CsA) on contagious bovine pleuropneumonia Disease Development	O. Hübschle, CVO, Windhoek, Namibia
16:20 – 17:00	Discussions on CBPP vaccines/ molecular research	
17:30 – 19:00	Cocktails	Indonesia Room, 8th Floor Building B

TUESDAY, 7 NOVEMBER 2006 – PHILIPPINES ROOM, C 277/281
CBPP Diagnostics/Surveillance

Session Chair		**F. Thiaucourt, CIRAD/EMVT Montpellier, France**
09:00 – 09:20	IAEA coordinated CBPP projects	H. Unger, FAO/IAEA Vienna, Austria
09:20 – 09:40	CBPP: AU-IBAR perspective	Prof. A.A. El Sawalhy, AU-IBAR, Nairobi, Kenya
10:00 – 10:20	Coffee break	
10:20 – 10:40	The evolution of CBPP in southern Africa: 2003–2006	Fred. L. Musisi FAO/RIASCO-TCEO, Johannesburg, South Africa
10:40 – 11:00	The current situation of CBPP in Zambia: Prospects for control	Jim Belemu FAO, Lusaka-Zambia

11:00 – 11:30	Discussions	
11:30 – 11:50	Control of CBPP in Tanzania: The implementation of a roll-back vaccination plan	Peter Njau MIFUGO Dar es Salaam, Tanzania
11:50 – 12:10	ALive CBPP research programme	Francois Thiaucourt, CIRAD/ EMVT, Montpellier, France
12:10 – 12:30	CBPP research in Portugal: new outlooks in the post-eradication era	Anna Bothelo, LNIV, Lisbon, Portugal
12:30 – 12:50	Discussions	
12:50 – 14:00	Lunch Break	

WEDNESDAY, 8 NOVEMBER 2006 – PHILIPPINES ROOM, C 277/281
Working Groups

Session Chair		**W. Amanfu, FAO**
	Recommendations committee	
15:30 – 15:50	Coffee Break	
15:30 – 17:00	Working Groups	

Working Groups

Session Chair		**W. Amanfu/J. Lubroth, FAO**
08:30 – 10:00	Working Groups Reporting	
10:20 – 10:40	Coffee Break	
10:40 – 12:30	Drafting of Recommendations	
12:30 – 14:00	Lunch Break	

Working Groups

Session Chair		**J. Domenech/J. Lubroth CVO/Chief Animal Health Service-FAO**
14:00– 15:00	Plenary, Finalization of Recommendations	
15:00–15:30	Closing	

The theme of the Contagious Bovine Pleuropneumonia (CBPP) Consultative Meeting is *"Control of CBPP: antibiotics to the rescue?"* Whilst this theme may appear provocative, it is apparent that a vigorous scientific debate and research are needed in answering some of the questions frequently asked on the therapeutic efficacy of antibiotics in managing CBPP disease. Antibiotic use in attempts to control CBPP is a fact of life especially in pastoral settings, contrary to official policy. It is for these reasons that the consultative meeting was held, in an attempt to provide the relevant platform for interactions on the use or none use of antibiotics in CBPP disease management within the context of available technical information on this subject matter.