REPORT OF THE

FIRST MEETING OF THE

FAO/OIE/OAU-IBAR CONSULTATIVE GROUP ON

CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

Rome, Italy
5-7 October 1998

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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INTRODUCTION AND OPENING SPEECH

Dr. Y. Cheneau
Chief, Animal Health Service, AGAH

It is my pleasure to welcome you all and thank you for having accepted to give up your valuable time to come and attend this first meeting of the CBPP Consultative Group; a joint undertaking of FAO and other major players in the field of CBPP, namely OIE and OAU.

As we are all aware, in the sixties there used to be regular meetings on the occasion of which mutual experiences were exchanged with regard to CBPP diagnostic techniques, control measures, etc., and related subjects were debated leading to recommendations that were then disseminated across the world. I am told the last gathering of this authoritative FAO/OIE/OAU expert panel dates back to 1971.

It is noteworthy that, concomitant to the eradication of CBPP from Australia, and the fact that the situation in Africa seemed to be under control, the interest of the scientific community and the regional and international animal Health Organizations was shifted towards priorities other than CBPP.

However, after almost 20 years of respite, CBPP has made a spectacular come back on two major African fronts: one in the east of the continent and the other one in the south. At the same time we are witnessing a breakthrough of the unstable equilibrium which used to prevail in endemic areas probably due to the cessation of the rinderpest vaccination and therewith the weakening of the state of immunity in the cattle population that used to be conferred through the use of the rinderpest/CBPP combined vaccine.

This situation urged us to call upon all concerned parties and draw their attention to the newly increasing importance of CBPP throughout Africa. The present meeting, which seems to be very timely has a dual objective in my opinion: (1) to sum up the state of art with regards to current scientific knowledge on CBPP and underline prospects for improvement that can be expected in the foreseeable future and; (2) to seek consensus from all concerned that CBPP has become one of the major diseases in Africa (if not the most important), and therefore, all efforts should be directed towards implementing an effective and realistic strategy aiming at its control leading to regional eradication from Africa.

Your Group is expected to become the authoritative forum through which FAO, OIE and OAU member countries will receive advice on adequate control strategies; the scientists will be advised on relevant research avenues worth pursuing and the donor community and decision-makers will be advised on prioritization in terms of resource allocation.

I leave it to you to debate on whether your group should be institutionalized or not, at what interval your meetings should be convened and if it is relevant to have a CBPP standing committee. I can only assure you on behalf of FAO that CBPP is on the top of EMPRES priorities as it is one of the three Transboundary Animal Diseases for which the 1996 EMPRES consultation has advised FAO to play a strategic role in early warning, early reaction, enabling research and coordination.

I wish you well in this important meeting and look forward to reading the results of your deliberations in due course.

Epidemiology

Summary

CBPP is now the most important transboundary disease in subsaharan Africa. It continues to spread both within endemic and in hitherto disease-free areas. In 1997 for instance, over 26 countries reported one or more incidences of CBPP outbreaks. An examination of reported cases of CBPP over the past two decades demonstrates an alarming worsening situation both in terms of the number of outbreaks and the severity of disease.

Country by country status of CBPP was received. Some of the reasons for the worsening or deteriorating CBPP situations are:

- uncontrolled animal (cattle) movement;
- cessation of vaccination campaigns against rinderpest using combined CBPP–rinderpest vaccines;
- lack or insufficient resources allocated to disease control undertakings;
- changes of vaccine strains e.g. T_1SR vaccine has been suspected to be of low protective immunogenicity;
- changes in policy, e.g. institution of cost recovery schemes in vaccination exercises seems to have had negative impacts; and,
- decreased surveillance. In this regard it was pointed out that meat inspection has an important role to play as an inexpensive means of surveillance to assess the status of chronic disease in a country through abattoir examination of CBPP-like lesions in the lungs.

Methods for conventional and modern serological techniques for CBPP surveillance were discussed and it was stressed that the techniques should be standardized in different laboratories. It was recognized that there is an urgent need to collate information on CBPP to facilitate formulation of modules for the disease surveillance methods as a strategy of monitoring effectiveness of the disease control/surveillance processes.

Conclusions

There is an urgent need to control CBPP. This calls for the formulation of regionally coordinated disease control strategies. Meat inspection as a tool for disease detection should be integrated in the defined control strategies.

Causative agent

Summary

(a)   Taxonomy and molecular biology

The taxonomy of *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*SC) was reviewed including its host specificity and disease caused in relation to the members of the *Mycoides* cluster.

Work based on the study of DNA fingerprinting (IS*1296*), restriction fragment length polymorphisms (RFLP), biochemistry (substrate utilization, carbohydrate study), and antigenic profiling (Western blotting) seems to suggest that recent African and European isolates of *Mmm*SC fall into two distinct subgroups. However, the quantity of the work done so far is insufficient and there is a need to carry out a more detailed epidemiological study on the African isolates to further define the virulence factors as determined by modern molecular biology procedures. To understand the virulence factors better, a sound elucidation of the immunology of *Mmm*SC should first be undertaken.

(b)   Diagnostic techniques

(i)   Isolation and identification

Isolation and identification of *Mmm*SC is crucial in the diagnosis of CBPP. Procedures for sample collection, transportation, mycoplasma culture, cloning and standard identification by biochemical and growth inhibition tests were described.

The value of polymerase chain reaction (PCR)-based systems (and colorimetric product detection) were recognized as powerful diagnostic tools with high sensitivities and specificities. These could be used for the identification of *Mmm*SC directly from clinical samples and confirm isolated organisms. The procedure should be standardized and the technology transferred to regional CBPP laboratories in Africa.

Other antigen detection tests such as immunohistochemistry, if properly executed, could be robust tools, but are labour intensive. Antigen detection by sandwich ELISA was a possibility which should be explored.

(ii)   Serological techniques

Currently, serology was the method of choice for the laboratory-based, mass screening of populations to determine the status of the disease. It was stressed that in the course of the disease the initiation of detectable antibody production was not until two to three weeks after contagion contact. So, the early detection of infected cattle is theoretically impossible for a system based on antibody detection. Nevertheless, several systems were described with respect to their merits and shortcomings.

Agglutination tests were rapid but tended to give high proportions of false positives. Complement fixation test (CFT), which is the OIE reference test, was considered costly and difficult to standardize and had a tendency to give false positive results for unknown reasons, especially in non-infected bovine sera. Dot blot immunoblotting assays which used monoclonal antibodies had the drawback of difficulties in their validation. Enzyme-linked immunosorbent assay (ELISA) were easily standardized, had sensitivities similar to CFT but were less laborious. Latex agglutination tests (LAT), once standardized, offered the opportunity for pen-side tests.

(c)   *Mmm*SC genome, virulence and immunogenicity

Genetic analyses of *Mmm*SC carried out in the past formed the important basis of newer, more detailed studies which have led to the development of several PCR-based tests. They have also allowed the description of the taxonomic and phylogenetic status of *Mmm*SC within the *Mycoplasma* cluster and provided evidence which shows that this is a stable group

of organisms. The genetic basis of virulence is not known. Earlier studies have pointed out the importance of galactan in the capsule-like surface structure of *Mmm*SC and the haemolysin-like activity of these organisms. The presence of variable surface proteins on other pathogenic species of mycoplasma have been discovered and similar structures are also suspected to be present on *Mmm*SC. Five proteinaceous molecules were consistently found to be the most antigenically dominant. A metabolic difference in the utilization of glycerol was described between African and European strains which resulted in the enhanced production of peroxide by the African strains. Although the relevance of this observed difference is not known it is to be noted that oxidative damage to the host is one of the main facts of the pathogenesis by mycoplasmas.

(d)   Pathogenicity

Little was known of the pathogenesis of CBPP. Most of the current knowledge was derived from observations made from studies about 30 years ago. Several theories which have been formulated were discussed but the one which best fitted the observations of patchy stasis and necrosis of the lungs known as 'marbling', was the one which proposed the local blockage of the lymphatic system. The proposed mechanisms of the formation of particular reactions, such as Wilhelms and Arthus reactions, were described. It was clear that allergic and autoimmune reactions were part of the reasons for the disease syndrome of CBPP.

Conclusions

The PCR-based tests were powerful tools which could be used by key laboratories. The current serological tests, including the cELISA, were not capable of the detection of chronic carriers. Therefore, the threat of new introductions of CBPP into disease-free areas as a result of trade still remained. Vaccinated animals were not detected by this test. Rapid field tests were urgently needed. Studies of virulence factors, immunogenicity, and pathogenicity at a molecular level should be encouraged, and would result in the better understanding of the disease and the development of efficacious reagent for the control of the disease.

Vaccines and vaccination

Summary

It is evident that strict cattle movement control is not be feasible in most of the African continent at present. A more realistic option remains mass vaccination with high coverage using good quality vaccines. Certification of quality by PANVAC has been implemented to ensure vaccine conformity with the OIE requirements for such products. However, several issues of quality and consistency were pointed out which must be resolved.

Clearly-defined, internationally-agreed seed strain for CBPP vaccine in Africa was required. Current tests were not able to distinguish vaccine strains from field or contaminant *Mmm*SC strains. Difficulties were experienced in the achievement of high mycoplasma yields and methods of increasing yield, perhaps by the of use of fermenters, were suggested. Process quality control was difficult because tests which conclusively discriminate wild strains of *Mmm*SC from those employed as vaccine strains were not available. In the field, problems in potency were reported and, although these could have been due to instability and misuse, a revalidation of the immunogenic potency and efficacy of the current vaccine was required. Cheaper methods of assessing the potency were required. A more stable product

may be achieved by the optimized lyophilization cycles and/or by the addition of a stabilizer. More heat resistant vaccines or formulations were necessary. Reports of adverse effects, mainly reactions at the site of inoculation, were received from the field and efforts must be made in making the current vaccine strains less virulent. New technologies were being applied which may address some of these problems.

Eradication of CBPP is possible only by stamping out. Progressive control by vaccination leading to eventual eradication depended on coordinated actions directed towards the interuption of the transmission cycle. Efforts from community-based animal health workers, and private sector contractors, which catered for nomadic populations were required. The projected efficiency of such management strategies could be assessed by the use of mathematical modelling. The decrease in rinderpest vaccination with Bisec appeared to follow an increase in CBPP in some countries such as Ghana, but in many other areas no clear trend was seen, and in some other vaccinated areas CBPP incidence was stable. Withdrawl of the PARC-funded bivalent vaccine may have the consequent effects of poor coverage for CBPP vaccination for economic and technical reasons. Production and use of monovalent vaccines was not under the control of PANVAC in some cases.

Effective epidemiology relies on accurate data and effective diagnostic tests are essential. Increased surveillance was required to establish prevalence and incidence supported by standardized diagnostic tests. Potentially powerful tests such as PCR-based detection systems should be standardized and their performance established. Assays of known predictive values should be used in diagnosis which would result in accurate evaluations of eradication strategies.

Conclusions

Vaccination was the only control measure available for many African nations. Correct production and use of quality controlled products was essential. Cost relief was necessary in some areas and community owned animal welfare schemes were necessary for the day–to–day administration of control efforts. Newer technologies were necessary for the quality assurance of vaccines and diagnostic tests.

Research needs

Summary

The current thrust of reseach is towards the molecular characterization and description of *Mmm*SC. Considerable efforts are being made to sequence the entire genome of the organism and this task should be complete in 1999. Genetic technology was also being used to describe the immunoreactive components of *Mmm*SC especially those with suspected actions on the host immune system. Information gained from this will assist in the development of better diagnostic tests, vaccine development, and tailored chemotherapeutic agents.

Research in the field of vaccination with immune stimulating complexes, ISCOM, were underway and were reported to produce immunity in experimentally infected mice and cattle at doses much less than those of current live vaccines. However, recent field trials were not successful and further experiments, including dose response investigations, are required.

The role of antibiotics in the control programmes required re-examination. The thought that the use of antibiotics in CBPP-affected cattle lead to the formation of sequestra and thus the source of infective material creating new infective foci needs to be substantiated. *Mmm*SC was susceptible to a number of chemotherapeutic agents. The rapid and spectacular effects of antimicrobial therapy would be an advantage in the control of CBPP, especially where vaccination and stamping out were impractical or impossible, and its inclusion into control strategies requires assessment.

Research in these areas should be coordinated and several European Union-funded projects in the FAIR and COST schemes provided arenas for information exchange and interaction. Members of African nations were encouraged to participate in these meetings where possible. Further research was required into the pathogenesis and immunology of *Mmm*SC and some initiative for collaborative projects was invited from African nations.

Conclusions

Research was an essential part of the maintenance of up-to-date diagnostic and therapeutic reagents. Much basic knowledge and some essential knowledge on the causative organism and its effect on the host were lacking. Biological basis of pathogenesis and the protective immune response needed urgent research.

Surveillance and control strategies

Summary

A region–by–region update of the current situation was heard. Alarming spread of the disease in West Africa, especially in Ghana, was the result of increased trade and cattle movements. Cattle movement was reiterated as the cause of old and new outbreaks in Eastern Africa and Southern Africa. Coordinated control through PARC-established laboratory and field structures was suggested. The recent Botswanan outbreak and its prompt control by stamping out was described. Movement control through border fences and inter-zonal fences was effective. Surveillance through concensus testing and later through random sampling was effective. Regional collaboration was essential in transboundary control, and coordinated use of funding, hightened public awareness, and good political will were essential items for the control of CBPP.

CBPP outside Africa was reviewed and, in Europe, Northern Portugal was the last area which still reported the disease. Staged stamping out is currently being applied there for the eradication of the disease. The Gulf States reported occasional imported problems, and Yemen, Bangladesh and Myanmar may have some cases, but reports were difficult to verify.

The economic considerations of CBPP were considerable and included direct and indirect effects. The direct effects such as trade losses, were relatively easy to calculate but some of the indirect effects such as the run-on and social consequences were difficult to assess. Thus, control strategies were to consider as far as possible as many of these factors for the estimation of costs and benefits.

The OIE Pathway, which describes the steps to be taken for the declaration of freedom from disease, was reviewed. For countries where vaccination against CBPP is not practised, an accelerated Pathway was available.

Conclusions

CBPP is a problem of considerable proportions both economically and socially. Measures for its control will depend on accurate data collection, monitoring, and strategies which take into account all the various aspects.

FAO/OIE/OAU–IBAR concepts and guidelines for CBPP control

The most effective control measures for CBPP, from early recognition to slaughter of exposed cattle, may not be met in certain situations. In these cases an alternative approach is the vaccination and quarantine of such animals. Total cover must be the aim of any vaccination strategy for CBPP which will require repeated administrations for several years until eradication is demonstrated, and this sustained effort may be difficult to realize.

The EMPRES concept on the control of CBPP in Africa especially for Eastern and Southern Africa envisages the division of the region into three epidemiological categories: (1) free areas, where surveillance and emergency preparedness are planned; (2) infected areas, where intense control by stamping out or vaccination and quarantine are instituted; and (3) cordon sanitaire, where a defined buffer zone between clean and infected areas are maintained.

In the African continent two cordons are envisaged; an Eastern Buffer Zone covering the international borders between Tanzania and Zambia, Malawi, Mozambique, and the area of DRC immediately to the west of lake Tanganyika (Ituri, Nord and Sud Kivu); and, a Western Buffer Zone stretching from the Atlantic Coast across northern Namibia, northern Botswana and western Zambia (Figure 1).

These buffer zones will have two components: (a) a surveillance zone at least 50 km deep covering the disease-free side of the international border in which no vaccination will take place while intensive surveillance and animal movement control will be enforced; and, (b) a control zone at least 100 km deep covering the infected side of the border immediately adjoining the surveillance zone. Control will be maintained in this area by: intensive vaccination, surveillance and movement control.

Zoning should also be devised within the control zone to include: (1) infected zone (active foci), where quarantine must be strict and coupled with vaccinations at months 0, 3, 9, 21, then annually; (2) high risk zone, where surveillance and vaccination at months 0, 6, 18, then annually are done; (3) low risk zone, where surveillance and annual vaccination are done; and, (4) minimal risk zone, where no vaccination is practised, and early warning systems are in place.

For West Africa, a draft strategy was devised in Nouakchott, Mauritius, in February 1998. The sub-region, comprising 15 countries from the Atlantic Ocean to the Niger/Nigeria to Chad/Cameroon borders, was divided into three groups of countries.

Group 1 contains countries not declaring CBPP, such as Senegal and Gambia, who should enter the OIE pathway towards CBPP freedom after ceasing vaccination (last campaign this year) and declare themselves provisionally free of CBPP. A cordon sanitaire must be established on the border of Senegal and other neighbouring countries which comprises two zones. A surveillance zone, 50 km deep where no vaccination should take place, should be established first. Should CBPP break through in this zone, all of the affected herd(s) should be destroyed and their owners compensated. The source of infection must be determined and contact herds examined for CBPP. If other herds are found infected, the same should apply unless it becomes obvious that such a strategy is no longer viable. The surveillance should then be transformed and extended to form a control zone 100 km deep where mass vaccination is implemented annually for at least the next 5 years (free vaccination encouraged). A new surveillance zone ought to be delineated outside the control zone.

Group 2 contains countries at risk (recipients of infection), and are mostly countries on the coast such as Nigeria, Benin, Togo, Ghana, Côte d'Ivoire, Liberia, Western Guinea, Sierra Leone and Mauritius. In these countries compulsory vaccination must be instituted upon departure in transhumance, and all vaccinated animals to be ear-tagged. When new outbreaks occur, the whole population at risk should be re-revaccinated at government's expense. The OIE/FAO/OAU–IBAR recommendation of vaccination at 0, 3, 9 months then annually should be followed. Slaughter with compensation should be encouraged, but it is hoped that in the near future the use of cELISA may enable reconsideration of this constraining scheme.

Group 3 contains countries where CBPP is endemic such as Mali, Guinea, Eastern Guinea Bissau, Burkina Faso and Niger. A focal area of endemic infection is present in the delta of Central Niger where animals from Mali, Burkina Faso, Niger and Mauritius gather frequently. Another area of endemic infection seems to cover East Guinea and Guinea Bissau. Also, Southwest of lake Chad i.e. Nigeria, north-western Cameroon and Chad is an endemic area thus this strategy must be extended to other countries to the West and Central Africa. In these areas vaccination must be compulsory, generalized, coordinated and repeated for at least over 5 years. It may be possible to provide vaccine free of charge, but compliance must be ensured with punitive policing measures if necessary. Substantial commitment is also required from the part of veterinary services in these endemic areas.

Countries belonging to Groups 2 and 3 should embark and commit themselves to the OIE Pathway towards CBPP eradication. The definition of zones (according to FAO terminology as explained above) should be done in the transition period during which vaccination campaigns are carried out.

Figure 1. Proposed buffer zones for the control of CBPP in Africa

SUMMARY OF RECOMMENDATIONS

Preamble

The recent alarming spread of CBPP has highlighted the decreased control of the disease throughout Africa. The reasons for this include shortcomings in the basic understanding of the disease and the implementation of effective surveillance and control programmes. This prompted FAO together with OIE, FAO/IAEA and OAU–IBAR to convene a joint meeting of specialists to review the current situation and to suggest actions for the improvement of this situation. The meeting was held at FAO HQs in Rome from 5 to 7 October 1998. The main outcome of the meeting was the recognition of the need for a CBPP Consultative Group (CG) to continually update the current knowledge and advise on the progress of improved strategies for the control and eradication of the disease. The following recommendations were made:

Recommendations

1   Strategy development of an integrated and coordinated regional control programme

1.1   The FAO and its partners should formalize the Consultative Group (CG) in order to ensure its continuation as a body that will provide guidance on CBPP control and research. Standing members of the CG would include FAO, OIE, OAU–IBAR, FAO/IAEA, the newly recognized World Reference Laboratory for CBPP and other appropriate collaborating centres and individual specialists. The Secretariat will be hosted by FAO.

1.2   The CG should prepare, in consultation with concerned countries, a strategic plan for the improved control of CBPP. A fully comprehensive programme for the progressive control of CBPP throughout the world, with special focus on Africa is to be implemented. The ultimate goal is eradication following the OIE pathway in accordance with the International Animal Health Code.

1.3   It was recognized that the FAO/EMPRES group possesses the required skills to advise on standardized surveillance systems at continental and national levels, and to establish guidelines for these. It was recommended that these guidelines should be implemented through regional networks such as those already existing in OAU–IBAR and SADC. In conjunction with data from socio-economic studies the baseline data generated would be used for the economic analysis and disease modelling of optional control strategies.

1.4   It was recognized that there is a shortage of funds for CBPP control at the regional and, in most cases, national levels and that most future initiatives will require donor assistance for funds and resources. It was, therefore, recommended that the newly established CG should invite donors to participate in CG meetings as observers.

2 Surveillance, modelling and economics

2.1 FAO and partners should prepare appropriate standards, supported by clear documentation, and training materials, for abattoir surveillance, serological surveillance, and clinical disease search.

2.2 FAO/IAEA in collaboration with their partners should prepare appropriate standards, supported by clear documentation and training materials, for laboratory diagnosis.

2.3 National CBPP committees through their epidemiology networks will generate baseline data for economic assessment of the disease to determine its true costs and impact. Furthermore, disease modelling is recommended to investigate the efficacy of ongoing control programmes and develop other cost effective control options.

2.4 Detailed field and laboratory studies should be made to further elaborate the epidemiology of CBPP. In particular:

- The risk of transmission from recovered cases/lungers — breakdown to transmission.
- The effectiveness of chemotherapy and its effect on development of carrier state.
- The possible reversion to virulence of live vaccines.
- The persistence of infectiousness after recovery from disease and sub-clinical infection.

2.5 The development of robust sampling and transport methods for antigen and antibody detection systems should be undertaken.

2.6 The development of new molecular typing systems should be actively encouraged and their results continually related to accurate field surveillance data in order to establish reliable and meaningful molecular epidemiology.

3 In–country capacity building to support the strategy

3.1 Public Veterinary Services should have a strong central authority supported by clear implementable legislation with adequate human and financial resources.

3.2 A national CBPP committee should be established in each concerned country. It should comprise among its members a veterinarian with recognised expertise in CBPP having the responsibility of liaising with the CG and the appropriate regional Organizations (e.g. OAU–IBAR, SADC etc.).

3.3 The public sector should be encouraged to examine the feasibility of sub-contracting vaccination against CBPP to the private sector, including NGOs and veterinary supervised community based animal health workers. Also it is proposed that governments could subsidize the supply of vaccine in order to increase vaccination coverage.

3.4 National Authorities should endeavour to deploy as necessary trained manpower for CBPP diagnosis, surveillance, research and control.

3.5 Efforts should be made to establish CBPP research laboratories on a regional basis. The allocation of the laboratories should be determined following an evalution exercise of the present facilities in Africa. Adequate finance, equipment and materials should be provided to the laboratories for efficient functioning. Also, an enabling environment should be provided to retain scientists and the support staff.

3.6 It is proposed that when budgets for CBPP control are being established a defined proportion (5 to 10 percent) be allocated for research purpose.

3.7 As far as possible, research on CBPP should be carried out in African institutions.

3.8 Also the veterinary curricula should emphasize CBPP, and continuing education should be encouraged to up-date field veterinarians on the latest knowledge in diagnosis, epidemiology and control. Additionally, there should be access through the CG Secretariat to a complete collection of archival and recent published reports and studies pertaining to the control of CBPP.

3.9 Information and communication systems should be put in place to monitor the efficiency of the adopted strategy. This will enhance general awareness and sensitize politicians, administrators and breeders on the importance of CBPP control/eradication.

4 Vaccine production, research and quality assurance

4.1 This meeting noted that the use of KH3J in CBPP vaccination was no longer recommended by the OIE.

4.2 The use of bivalent rinderpest–CBPP (Bisec) vaccine is no longer advisable.

4.3 On account of field observations which have cast some doubt on the immunogenicity of T_1SR, the use of this strain in CBPP vaccines should await the results of conclusive cattle efficacy trials. For the time being T_144 remains the recommended seed strain. A reference seed lot which has been jointly produced and tested by PANVAC and EMVT can be obtained from PANVAC.

4.4 Specific *in vitro* tests for definitive vaccine seed strain identification are needed.

4.5 The OIE manual section on the vaccine culture inoculation needs to be revised to avoid the risk of inadvertent cloning of vaccine seed culture. One way may be to inoculate the whole contents of a vaccine seed vial directly into 100 ml of medium. After incubation this can then be used to inoculate vaccine bulk cultures.

4.6 Noting PANVAC's results of 957 titrations on 319 CBPP vaccine batches from 10 different producers, the meeting considered that the mycoplasma content for the 100 dose pack is only marginally above the OIE minimum requirement. Consequently, it

is recommended that vaccine manufacturers should strive to limit the prescription of the number of doses per vial, as currently constituted, to only 50.

4.7 It was also noted that there is a need for vaccine packs of 10 and 20 doses, especially for use in small-scale holdings and pastoral areas.

4.8 Means of increasing mycoplasma final titre should be investigated, e.g. through the fermentation process. Such products should be fully quality controlled including testing in cattle before they are adopted for routine use.

4.9 Procedures to improve the thermostability of vaccines should be defined e.g. optimized freeze-drying cycles using appropriate excipients or stabilizers.

4.10 Research aimed at the development of vaccines of defined antigenic or genetic character using conventional and/or recombinant DNA technology should be encouraged. Such vaccines would be expected to be less reactogenic than the current $T_1 44$ and to be able to confer regularly an immunity which lasts longer than one year.

4.11 There is a need for a wider use of experiments in cattle, in Africa, for evaluation of various aspects of vaccines including determining the immunizing dose, route of administration, onset and duration of immunity and evaluation of various formulations.

4.12 Research on the application of the ISCOM technology which now offers an opportunity to study antigen delivery systems, adjuvant formulations, definition of antigenic determinants and a re-examination of the killed vaccine alternative should continue to be supported.

5 Standardization and improvement of diagnostic procedures

5.1 National Laboratories should strive to obtain recognition of proficiency through the use of standardized assays (Standard Operating Procedures), well defined controls, and compliance with external quality assurance schemes and national or international veterinary laboratory accreditation scheme.

5.2 The ability to both detect the causative agent (or part of it) or antibody to it at the cow-side is considered important at the herd level for CBPP control and eradication programmes. A number of assays are under evaluation and support should be given for their further evaluation and validation.

5.3 It is essential that national laboratories have an ability to isolate and identify the causative agent of CBPP. Standardized procedures for the culture and identification (including descriptions of the broth and reference sera) should be prepared and distributed. National laboratories in infected countries should ensure that the necessary reagents and skills are available to ensure that this can be carried out.

5.4 Whilst PCR is an invaluable tool both for initial confirmation of a diagnosis and for more long-term molecular epidemiology studies it is difficult to standardize and

quality assure. It is recommended that as a minimum the African CBPP Reference Laboratories should have an ability to carry out PCR for CBPP.

5.5   Capture ELISA could prove a useful method for rapid antigen detection at the laboratory level and studies should be undertaken to develop and validate such an approach.

5.6   The CFT is the prescribed test recommended by the OIE. Nevertheless, it has significant limitations regarding sensitivity and specificity, and is difficult to quality assure and rather costly. Initial studies on a competitive ELISA (cELISA) show great promise and the validation work on the cELISA and its comparison to the CFT should be completed as a matter of priority. If this assay shows equal or greater sensitivity and specificity to the CFT it should be adopted by the OIE as a Prescribed Test and an internationally standardized kit should be made available to infected countries in Africa.

5.7   It is likely that even with the cELISA some false reactions will occur. It is recommended that the immunoblot assay (IBT) be used as a confirmatory test and in the validation of the cELISA positive sera should be re-tested using this assay.

5.8   Antibody assays that clearly identify vaccinated animals, and separately, infected animals will be vital for surveillance studies. Current assays (CFT, cELISA) do not achieve this and every effort should be made to ensure that such assays are developed as a matter of priority. Equally, an assay that correlates with immunity (cell-mediated) is vital for vaccination purposes use and should be developed.

5.9   The designation by FAO and OIE of a CBPP World Reference Laboratory and the identification of Regional Reference Laboratories is considered essential.

6   Investigations into the pathogenesis / immunology of CBPP

It was recognized that there were at least two major areas that require further investigations i.e. (1) how initiation of the infective process takes place and; (2) how to explain the clinical course of the disease. This requires a better understanding of:

- the cellular and humoral immune responses to *Mmm*SC and components of *Mmm*SC;
- the mechanisms of the pathogenic process especially the early interactions between the organism and the host;
- the role of toxins and extracellular components in pathogenicity and the importance of autoimmunity in the disease process;
- the relevant protective responses to *Mmm*SC antigens with a view towards the development of more effective vaccination strategies.

Therefore:

6.1 Investigations by a multidisciplinary team need to be conducted to (1) map the immune response including the expression of cytokines and lymphokines etc.; (2) check the response of the host to separated fractions of the causative agent e.g. carbohydrates, surface proteins; and re-examine the mechanisms of Willems and allergic and/or inflammatory reactions.

6.2 The CG secretariat should request the International Livestock Research Institute (ILRI) to incorporate research on CBPP pathogenesis and immunology in their programme (technical advisory committee to CGIAR to be contacted for immediate attention).

APPENDICES

FIRST MEETING OF THE FAO/OIE/OAU–IBAR CONSULTATIVE GROUP ON CONTAGIOUS BOVINE PLEUROPNEUMONIA

AGENDA

Chairman: **W. Masiga**

Rapporteurs: **J. B. Bashiruddin**
J. Litamoi

Monday, 5 October 1998

9.15 to 9.30: Opening address
Y. Cheneau, Chief, AGAH

9.30-10.00: Presentation of the Consultative Group and its relevance to EMPRES mandate
M. M. Rweyemamu

10.30-12.30: **First session: Epidemiology**

- Present situation of CBPP in Africa and epidemiological trends
W. Masiga

- Meat inspection as a tool for CBPP surveillance
F. G. Santini

- Conventional and modern serological techniques for CBPP surveillance
F. Thiaucourt

- TADINFO for CBPP
R. Paskin

- Discussions

14.00-17.00: **Second session: Causative agent**

- Taxonomy and molecular epidemiology
J. B. March

- Diagnostic techniques
R. A. J. Nicholas

- Large-scale cultivation of *M. mycoides* subsp. *mycoides*; freeze-drying and other methods of conservation
J. Tulasne (F. Thiaucourt)

- *M. mycoides* subsp. *mycoides* genome; virulence and immunogenicity
  J. B. Bashiruddin

- CBPP pathogenesis
  A. Provost

- Discussions

Tuesday, 6 October 1998

8.30-10.00:   **Third session: Vaccines and vaccination**

- Vaccine quality issues in Africa, including safety and efficacy
  J. Litamoi

- Vaccination requirements and logistics
  P. Roeder

- Impact of withdrawing rinderpest vaccination on CBPP control
  W. Masiga

- Discussions

10.30-12.30:   **Fourth session: Research needs**

- Mycoplasmology
  J. B. Bashiruddin

- Immunity and vaccination
  B. Morein

- Diagnosis and surveillance
  M. Jeggo

- Role of chemotherapy
  A. Benkirane

- Organization and coordination of research
  M. Rweyemamu and F. Thiaucourt

- Discussions

14.00-17.00:   **Fifth session: Surveillance and control strategies**

- West Africa
  M. Kané

- Eastern Africa
  W. Masiga and P. Rossiter

- Southern Africa
  P. Sinyangwe

- Botswana
  K. Masupu

- CBPP outside Africa
  P. Roeder

- The OIE pathway
  A. Provost

- Economic considerations for planning CBPP control programmes in Africa
  J. Otte

- Discussions

Wednesday, 7 October 1998

Developing FAO/OIE/OAU concepts and guidelines for CBPP control

- Towards an FAO/OIE/OAU Manual / Guideline for Progressive Control of CBPP in Africa
  A. Benkirane

- Drafting of recommendations and outline of CG publication

12.00   Closing ceremony

INDIVIDUAL PRESENTATIONS

THE PRESENT SITUATION IN AFRICA AND EPIDEMIOLOGICAL TRENDS

Walter Masiga, Paul Rossiter and Rene Bessin

Summary

Contagious bovine pleuropneumonia (CBPP) is present in at least 27 countries in equatorial, central and southern Africa and poses a major threat to the continent's cattle. In the past five years there have been alarming epidemic incursions of the disease into previously disease-free countries in east and southern Africa. In many of the other infected countries where the disease is endemic or sporadic there are reports that the incidence of the disease is also increasing.

1   Introduction

In Africa, disease remains one of the principal causes of the poor performance of the livestock sub-sector and of the ever widening gap between the supply and demand of meat and milk in the continent. Contagious bovine pleuropneumonia (CBPP), caused by *Mycoplasma mycoides* subsp. *mycoides* SC (small colony, bovine biotype) (*Mmm*SC) is present in west, central, east and parts of southern Africa but not North Africa. Where it occurs it is frequently a major constraint to cattle keeping and for this reason is considered the most serious animal disease in Africa together with rinderpest. At present, CBPP is spreading to countries where it had been previously controlled or eradicated and has caused heavy economic losses. The death of livestock in Africa has been estimated to cost 2 billion US$ per year.

1.1   Historical situation

Importation of cattle from The Netherlands introduced the disease into South Africa in 1854 from where it spread to other neighbouring countries in the region. Zimbabwe eradicated the disease in 1904, South Africa in 1924 and Botswana in 1939. Namibia and Angola have remained infected to this date and the infection was reintroduced into Botswana in 1994. Northern African countries have been infected only on a sporadic basis, the most recent being Egypt in 1972 where it was rapidly eradicated.

The origin of the disease in Central, West and East Africa is uncertain. It has been suggested that zebu cattle introduced the infection when they first migrated into the African continent (Dr. A Provost; personal communication) but there is a possibility that it was introduced into East Africa from India by the army of Field Marshall Napier when he invaded Ethiopia in 1867 to 1868. One of the first European explorers in East Africa, Joseph Thomson, witnessed a severe epidemic of CBPP in Maasailand in 1883.

Today CBPP is widespread in Africa and also occurs in Southern Europe (Spain and Portugal), parts of Asia (India and Bangladesh) and the Middle East (Kuwait).

2   An overview of the incidence of CBPP in Africa 1976 to 1997

A number of important meetings on animal health in Africa during the past three years have emphasized that CBPP is the most serious "transboundary" disease affecting the continent's

cattle. This is based upon both the geographical expansion of infected areas, with consequent epidemics, and the increased incidence in the endemic zones.

2.1   The geographical distribution of CBPP in Africa in 1997

The geographical distribution of twenty seven countries infected with CBPP in Africa are shown in Figure 1. There are discrepancies sometimes between the numbers of countries reporting CBPP each year, and between official and unofficial reports of the disease. Since the number of countries reporting the disease in any one year can therefore be misleading it was decided to examine the number of countries reporting the disease over the past 23 years, since the conclusion of the JP15, in order to build up a more detailed picture of the overall trend in reporting.

2.2   The changing incidence of CBPP in Africa

The number of African countries reporting CBPP during the past 23 years to the Office International des Epizooties, the Food and Agriculture Organization of the United Nations, or to the Inter-African Bureau for Animal Resources of the Organization of African Unity is summarized in Figure 2.

Figure 1.   The geographical distribution of CBPP in Africa, 1997

The 27 countries shown as infected with CBPP (shaded) on this map were included because of official reports and reported from other reliable sources. No reports were received for Equatorial Guinea, Gabon, Gambia, Guinea Bissau, Liberia and Sierra Leone.

Figure 2.   The number of countries reporting CBPP from 1976 to 1998
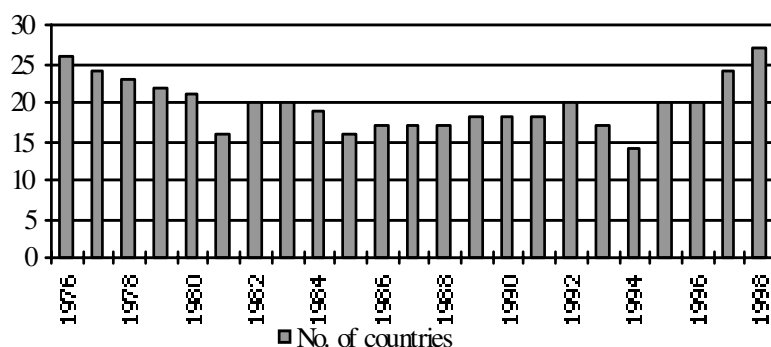
These annual disease reports have limitations and the data should be interpreted accordingly; for instance the sudden drop in the number of countries reporting CBPP in 1994 is because only half of the countries that usually report to OIE did so that year. Nevertheless, the data does appear to demonstrate trends in the incidence of CBPP in Africa during the past two decades. Overall it would appear that the number of countries now infected with CBPP is similar to what it was 23 years ago. In the interim there was a steady decline in reporting countries throughout the late 1970s and early 1980s, when many countries would have been using combined vaccination against rinderpest and CBPP, followed by a steady increase to present levels in the 1990s. Thus, there appears to have been a definite recent increase in the distribution of CBPP in Africa though the disease enjoyed a similar widespread distribution in the past. Clearly, although this aggregated data gives the continental trend it obscures most of the underlying changes and true epidemiological picture. In particular it does not show which countries are affected and to what degree. In addition, some observers have been concerned that the reported increased incidence in the previously endemically infected countries may have been over-emphasized in order to stimulate improved internal and external funding for hard pressed state veterinary services. To investigate the situation further the number of outbreaks of CBPP and, in some instances, related mortalities, in the affected countries during the past eleven years are recorded in Table 1.

These data confirm recent increases in the incidence of CBPP in several countries including Burkina Faso and Ghana in West Africa, Ethiopia and Tanzania in East Africa, and Botswana, Namibia and Zambia in Southern Africa. However, there are a number of countries, especially in West Africa, for which the officially reported data does not yet support their assertions of increasing incidence of CBPP.

3   The current CBPP situation in African countries, 1997/98

Angola: The South of the country is endemically infected and is a constant source of infected cattle to all neighbouring countries.

Botswana: In 1994 Botswana was re-infected with CBPP for the first time in 45 years by cattle returning from Angola and Namibia. As a result, all cattle in Ngamiland were slaughtered in 1996 and the owners compensated for their losses. Following the success of this operation and the favourable results of intensive clinical and serological surveillance

conducted in the region adjoining the infected zone, the country declared provisional freedom from CBPP in January 1997.

Burkina Faso: The disease is endemic and reported figures indicate that the incidence of new outbreaks is increasing.

Burundi: CBPP entered in Burundi in January 1997 and is believed to be still in the country.

Cameroon: After recording no CBPP in 1995, Cameroon experienced one outbreak in 1996 and two outbreaks in 1997.

Central African Republic: During the 12th PARC West and Central Africa Coordination Meeting in Accra, September 1998 it was reported that CBPP is present in some localities in this country.

Chad: Five suspected outbreaks were investigated but not confirmed.

Côte d'Ivoire: Ten outbreaks of CBPP were reported. A total of 1,573 cattle were infected of which 127 died and 44 were slaughtered.

Democratic Republic of Congo: There are unconfirmed reports that CBPP is now affecting cattle in the East of the country bordering Rwanda and Uganda.

Egypt: Egypt officially declared freedom from CBPP on 15 February 1976.

Eritrea: There are no reports of CBPP.

Ghana: In 1997, 50 outbreaks of CBPP were diagnosed, compared to five in 1996 and only one in 1995. The spread of the disease is considered to be associated with illicit cross-border livestock trade and introduction to village herds of young bulls and heifers purchased from cattle markets outside the country.

Guinea: The disease has been endemic in Eastern part of Guinea, Upper Guinea, for many years and a cordon sanitaire had successfully prevented it spreading to cattle west of the cordon. Unfortunately, the cordon was broken in 1995 when three outbreaks were detected in the disease-free zone. It is possible that this was due to new trade stock movements caused by the wars in Sierre Leone and Liberia. New outbreaks of CBPP continued to appear in West of the cordon in early 1997, originating from the endemic zone of Upper Guinea especially through the livestock market of Dagomet.

Kenya: The disease is endemic in the North and Northeast of the country from where it periodically escapes through uncontrolled cattle movement to cause epidemic outbreaks in the Centre and South of the country. Narok District which is contiguous with neighbouring Ngorongoro district of Tanzania is endemically infected. Nairobi is the main cattle market in East Africa and during 1997 outbreaks associated with the movement of tradestock occurred at two locations close to the city.

Mali: The disease is endemic in the country and reportedly increasing in incidence though this is not yet reflected in the reported number of outbreaks and mortalities.

Mauritania: After an absence of some years, outbreaks were reported in 1995 and 1996 and the incidence of the disease is reported to have increased in 1997.

Namibia: The northern part of the country is enzootically infected after the disease was re-introduced from Angola in 1983. Very good control of the disease has been achieved using T₁44 vaccine and at least 600 000 animals have been vaccinated with no adverse reactions.

Niger: The disease is endemic and nine outbreaks were reported in 1997.

Nigeria: The disease is widespread and endemic in the pastoral areas.

Rwanda: In 1994 refugees returned from southern Uganda with infected cattle. These caused classic epidemics in refugee and resident cattle with high morbidity (up to 70 percent in some herds) and high mortality (20 to 50 percent) rates. The disease has spread through virtually the whole country and into Burundi.

Senegal: No disease has been reported from Senegal since 1992 although all surrounding countries are now infected.

Sudan: No confirmed outbreaks of CBPP have been reported since 1990. However, non-governmental veterinarians working in the south of the country often encounter the clinical syndrome.

Somalia: There have been no recent confirmed reports of the disease, which was endemic in the country.

Tanzania: CBPP was re-introduced into Ngorongoro District of Tanzania in 1990 and is endemic in the area. Cattle rustling moved the disease westwards in 1991 and back into western Kenya. In 1991 and 1992 outbreaks appeared in the Kagera Region to the West of Lake Victoria as the result of uncontrolled cattle movement from Uganda. Initial efforts at control were thought to have confined the disease to these two Northern infected zones but in 1994 CBPP was diagnosed in several locations in the south and west of the country. This rapid spread was reported to be due to uncontrolled livestock movement and inadequate disease surveillance, diagnosis and reporting. Currently at least 29 districts are infected including Mbeya on the border with Zambia and Malawi.

Uganda: The disease was confined to Karamoja until the civil war during which it spread throughout much of the country and also to Rwanda and Tanzania. Currently CBPP is widespread in the country and fourteen outbreaks were reported in 1997. National mass vaccination campaigns are being carried out and include a cost recovery component.

Zambia: Cattle returning from Angola re-introduced the disease to Southwest Zambia in May 1997. It continued to be reported during the following months but emergency control activities appear to have confined the disease to this part of Zambia. An immune buffer zone has been established along the northern boundary to prevent the disease entering from immediately adjacent infected areas of Tanzania.

Congo, Djibouti and Togo: Though recent reports are lacking, the disease is believed to be present in these countries.

4 Epidemiological trends of CBPP in Africa

The recent history of CBPP in Africa shows two distinct epidemiological trends; epidemic outbreaks of disease in previously disease-free areas, and, increased incidence of disease in endemically infected areas.

4.1 Epidemic outbreaks in previously disease-free countries

Outbreaks of CBPP in previously uninfected areas have occurred recently in Rwanda, 1994; Tanzania, 1990, 1992 and 1994; Botswana, 1995; Zambia, 1997; Burundi, 1997 and the West of Guinea, 1995. There are also unconfirmed reports of the Democratic Republic of Congo having been recently infected. These new outbreaks were accompanied by the typical high morbidity and high mortality rates associated with epidemic disease in highly susceptible populations. All of the outbreaks were caused by the uncontrolled entry of cattle from known infected populations and represent a failure of cordons sanitaires, both national and international disease surveillance and vigilance. The outbreak in Botswana was eradicated by slaughter of infected and in-contact stock at a very high cost, Pula 400 million (approximately US$100 million) to the country's economy. In Zambia the disease appears to have been confined to the western quarantine zone. Elsewhere, the outbreaks were not so effectively controlled and, unfortunately, CBPP is now endemic in Rwanda, Burundi and much of Tanzania from where it threatens Malawi, Mozambique and Zambia. This is one of the most unsatisfactory and worrying developments in animal health in Africa in recent times.

4.2 Increased incidence in endemically infected countries

Most countries within the previously infected zones of west and east Africa report a rising incidence of the disease and in some countries this is supported by published information on the numbers of new outbreaks. The basic reason behind this increase in disease incidence is diminished control due to a variety of factors. These include reduced funding for vaccination, possibly associated with the cessation of externally funded vaccination campaigns against rinderpest, changes in vaccines and vaccine usage, cost recovery for CBPP vaccination, and reduced disease surveillance.

5 Conclusion

In the past five years CBPP has enlarged its geographic distribution in Africa through a series of dramatic and highly expensive epidemics. In addition there is mounting evidence that the incidence of the disease is increasing throughout most of the endemic areas of west and east Africa. Clearly, it is essential for Africa's livestock industry that these trends be reversed. There must be no more escapes of infection from infected to non-infected areas, and national control of CBPP in endemically infected countries must be re-established. At present there is no regional or sub-regional campaign to advise, assist and coordinate these issues. If there is to be such a future campaign it is important that the true distribution and incidence of CBPP are accurately and rapidly established in order to develop appropriate control strategies and attract suitable funding.

Table 1. The number of outbreaks of CBPP and mortalities reported by African countries (from 1987 to 1997)

Country	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Benin	+	+	+	4		+	5	12	3	8	7_{75}
Burkina Faso	10*	21	7	9	4	6	8	7	29_{115}	28_{221}	29_{93}
Côte d'Ivoire	7_{41}	9_{77}	14_{80}	18_{197}	16_{120}	9_{79}	12_{68}	7_{19}	12_{48}	11_{27}	10
Ghana	10	6	4	5	2	+	2	3	1	5	50_{18}
Guinea	?	105	49	38	82	49	20	17	48_{79}	30_{9}	+
Mali	4	5	15	37	12	15	20	21	11_{294}	12_{47}	6_{83}
Mauritania	0	0						0	6	5	+
Niger			0	5	8	13	4	8	3_{13}	8_{23}	9_{3}
Nigeria	49	46	114	48	55	27	17	18	6		+
Sierre Leone	0	0	0	0	+	+	0	0	0	0	0
Senegal	0	0	0	0	0	1	0	0	0	0	0
Cameroon					1	1	1		0	1	2
Chad	1	+	0	0	1	0					+
Burundi										+	+
Eritrea						+	+	+	0	0	0
Ethiopia	96	1	+	3	6	1	+	10	14	55_{295}	18_{93}
Kenya	6	2	15	39	24	11	10	6	6	3	+
Rwanda								+	+	+	+
Somalia	3	2									+
Sudan	+	+	+	4	0	0	0	0	0	0	0
Tanzania			0	+	1	+	1	3	259_{2462}	41_{375}	57_{572}
Uganda	49	56	4	11	28	5	3	+	18	6	14_{100}
Angola	1	22	26	+	15	21	19	38	149	50	+
Botswana	0	0	0	0	0	0	0	0	45	0	0
Namibia	4	6	0	1	2	3	2	7	25	47	26_{46}
Zambia	0	0	0	0	0	0	0	0	0	0	2_{21}

N_{X}, x= reported mortality (number of animals that have died).

* The data is taken from official reports contained in the OIE World Animal Health Reports, in the FAO/OIE/WHO Animal Health Yearbooks, and in PARC reports. In addition to the 21 countries in this table, it is believed that Congo, Democratic Republic of Congo, Djibouti, Central African Republic, Somalia and Sudan are also infected. Twenty-seven countries in total.

MEAT INSPECTION AS A TOOL FOR CBPP SURVEILLANCE

F. G. Santini

1   Introduction

In the last 15 years, contagious bovine pleuropneumonia (CBPP) has caused serious problems in Europe (France, Portugal, Spain and Italy) where it is still present in some remaining pockets of infection. In Africa the disease, due to the difficult environment, the collapse of Institutions in many countries and civil wars, has invaded new areas of the continent (east and southern countries) considered free since the 1950s and even before; in many central or western countries CBBP may be considered as endemic. The epidemiological situation in Asia and in European eastern countries is still unknown but the suspicion of the presence of CBPP is frequently reported.

To prevent the introduction of the disease or to control it once detected, is a very difficult task for any Veterinary Service of the world: the reasons are clear (long incubation period, chronic carriers, silent symptomatology, very complicated laboratory test, modality of the transmission of the disease and pathogenesis still unclear, result of antibiotics and vaccine treatment very often useless). The choice of the eradication and surveillance programmes mainly depend on the livestock system and farmer participation and by the organization of the Veterinary Services, their ability in animal movement control and laboratory testing, their information system and contingency plans, their financial possibility, their meat inspection activity.

To eradicate or control the disease there are only 2 possibilities:

- test and slaughter and surveillance
- vaccination campaign and surveillance

To maintain the free status, risk analysis evaluation (Morley parameters, OIE) may help the majority of the countries that import cattle or are target areas for foreign transhumant herd; other countries where live ruminant importation is very high, Italy for example, utilizing the above parameters, should close the market with their principal commercial partner, France for example, (Giovannini *et al.,* 1997). Surveillance in CBPP free areas as well as in infected zones may be carried out using several methods based on clinical examinations, serological or other laboratory test, but meat inspection always represents the more specific tool to suspect the presence of CBPP. The possibility of receiving up–to–date information on CBPP in the commercially-linked, or neighbouring, countries and the knowledge of the quality of the Veterinary Services in these countries, may indicate the more appropriate actions to be taken in a surveillance programme.

2   Meat inspection activity in CBPP surveillance

Meat inspection is one of the principal duties of the Veterinary Services to control the safety of the animal origin food for human consumption. During this activity of the Public Veterinary Health, the control of the sanitary condition of the animals, particularly for the presence of zoonoses and transmissible diseases, allows the veterinarian to recognize

characteristic and specific pathological lesions in the organs. CBPP may be detected or suspected at abattoir or in any slaughtering area that the Veterinary Services may control. Everywhere, in field conditions and even in the worst bush situations, a sort of meat inspection is carried out by veterinarians, meat inspectors or sometimes by the farmers themselves. If they know CBPP, they can suspect the disease and inform the official veterinary office of the region.

Why is meat inspection, and not abattoir control or post-mortem examination, a tool for CBPP surveillance? Meat inspection has a more comprehensive meaning because it includes the quality, or not, of the organization of the Veterinary Services, their ability to recognise CBPP in any conditions during routine activity, their information and alarm system. In the following Scheme 1, the principal actions that should be taken in a meat inspection surveillance programme are summarized.

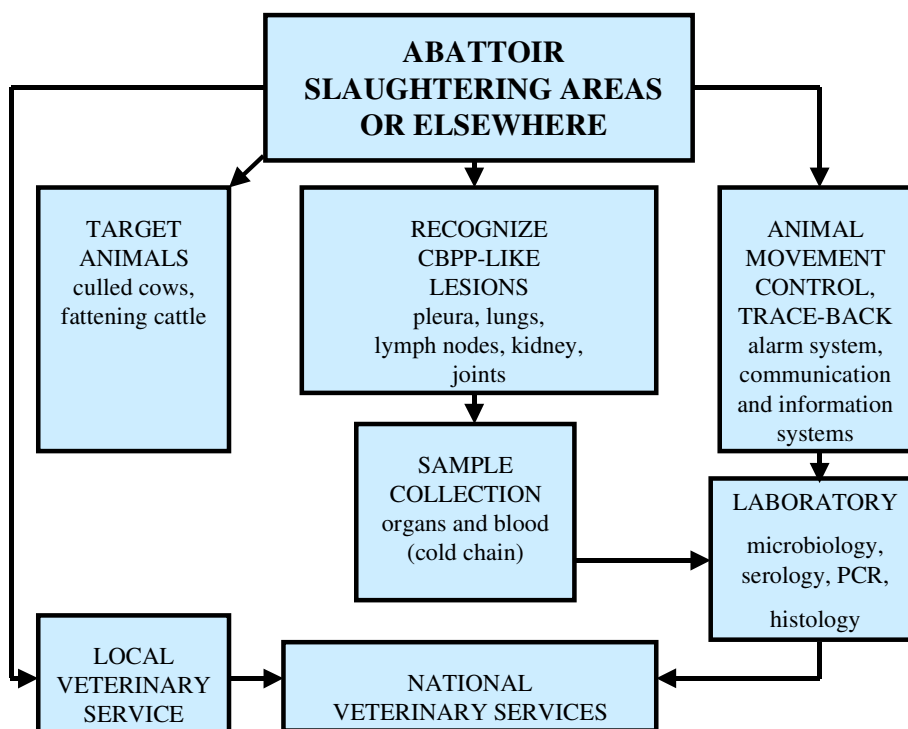## 3 CBPP meat inspection surveillance in free countries

3.1 *Condition: the country is situated in a free sub-region and has no commercial relationship with a hazard area*

Meat inspection is routinely carried out in almost all the countries of the world and, considering that pathological lesions of CBPP are very typical and specific, at the conditions that *inspectors have been refreshed on the disease*, this veterinary activity may be sufficient to confirm that the country is free.

Other conditions:

(1) Veterinary services may control slaughtering in the whole country;
(2) veterinary services are able to control animal movement;
(3) animal identification and farm registration have been implemented;
(4) abattoir data are recorded (information system);
(5) contingency plan against OIE list "A" disease is ready and efficient;
(6) communication network is available and efficient;
(7) laboratory techniques (OIE) are carried out.

Scheme 1

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                    ┌─────────────────────────┐
                    │       ABATTOIR          │
          ┌─────────│  SLAUGHTERING AREAS     │─────────┐
          │         │     OR ELSEWHERE        │         │
          │         └─────────────────────────┘         │
          │           ↙          ↓                      ↓
          │  ┌──────────┐  ┌──────────────┐  ┌──────────────────┐
          │  │  TARGET  │  │  RECOGNIZE   │  │     ANIMAL       │
          │  │ ANIMALS  │  │  CBPP-LIKE   │  │    MOVEMENT      │
          │  │ culled   │  │   LESIONS    │  │   CONTROL,       │
          │  │ cows,    │  │ pleura,lungs,│  │  TRACE-BACK      │
          │  │ fattening│  │ lymph nodes, │  │  alarm system,   │
          │  │ cattle   │  │ kidney,      │  │  communication   │
          │  │          │  │ joints       │  │ and information  │
          │  │          │  └──────────────┘  │   systems        │
          │  └──────────┘        ↓           └──────────────────┘
          │              ┌──────────────┐             ↓
          │              │    SAMPLE    │    ┌──────────────────┐
          │              │  COLLECTION  │    │   LABORATORY     │
          │              │ organs and   │───▶│  microbiology,   │
          │              │ blood        │    │  serology, PCR,  │
          │              │ (cold chain) │    │                  │
          │              └──────────────┘    │   histology      │
          │                                  └──────────────────┘
          ↓                                           │
  ┌──────────────┐   ┌───────────────────────┐        │
  │    LOCAL     │   │       NATIONAL        │        │
  │  VETERINARY  │──▶│  VETERINARY SERVICES  │◀───────┘
  │   SERVICE    │   │                       │
  └──────────────┘   └───────────────────────┘
```

3.2   Condition: the country is bordering with infected areas

When the country is bordering with infected areas, a specific surveillance programme must be implemented in the abattoirs where target animals (culled cows and fattening cattle) are slaughtered. During the recent epidemics in Italy, Bern University, Faculty of Veterinary Medicine, (Prof. Nicolet and Dr Vasari, 1994) carried out, in association with the Swiss Veterinary Services, an abattoir control to verify that the country was still free (Swiss cows CBPP positive were found in a Puglia outbreak in Italy). Before launching the programme, a short CBPP refreshment course was dedicated to all the Swiss meat inspectors. Abattoirs were chosen on statistical basis and a minimum number of samples were established to send to the laboratory; all CBPP-like lesions were processed and *Mycoplasma mycoides* subsp. *mycoides* SC was never isolated.

Veterinary inspectors must remember that:

(a) *In an infected area, sub-acute, chronic lesions and sequestra, of different size, are more frequently detected;*
(b) *very often only a single lung presents specific lesions;*
(c) *antibiotics may cover or change the typical macro aspect of the lesions at pathology examination;*
(d) *Pasteurella, other mycoplasmoses (Mycoplasma mycoides subsp. mycoides LC, Mycoplasma bovis), B. necrophorus, may frequently determine lesions similar to CBPP;*
(e) *young animals and calves very often do not present lesions in the lungs;*
(f) *the "classic" clinical manifestations are often absent or very light.*

Laboratories must be able to perform the official and recommended (OIE) diagnostic methods and particularly the more accurate microbiology test to isolate and identify *Mycoplasma mycoides* subsp. *mycoides* SC; considering that, during the slaughtering process, it is not complicated to collect blood, also the official serological test may be performed (CFT Campbell and Turner and, in future, the new ELISA). Other tests, such as PCR, demonstrate a very high specificity and sensitivity and may be used according to the limit of the test itself and the cost of the laboratory equipment.

During meat inspection, pleura, fibrin when present, limphonodes of the thorax, lungs, kidney and joint liquid should be selected, portioned and, in safety conditions, sent to the reference or better equipped laboratory of the country using cold chain facilities.

4 CBPP meat inspection surveillance in infected countries

4.1 *Condition: the country is carrying out a "test and slaughter" eradication strategy*

Italy is the only country where CBPP was recently eradicated in just a few years; meat inspection (abattoir control) was one of the principal tools used in the epidemiological surveillance to eradicate the disease. The strategy was based on clinical examination and serological testing before any bovine movement, serological random sampling, and abattoir control. Once a new outbreak was detected, a follow-up action included all the farms in both the protection and surveillance area where all >6, 12 month bovines were serologically tested and positive animals were slaughtered; all commercial liaisons of the infected farm were traced back (within 6 months). Meat inspection activity, carried out at national level, contributed to the detection of new outbreaks. *Controls in slaughterhouses were aimed at the validation of the whole system* through the examination of both fattening cattle and culled cows (Figure 1).

Figure 1. Detection of CBPP outbreaks

Herd type	Method of detection of CBPP outbreak			
	Serology	Pathology	Clinical	Unknown
Dairy	31	9	7	11
Fattening	1	18	2	5
Mixed	3	1	1	2
Trade	1	2	0	0
Total	**36**	**30**	**10**	**18**

The number of slaughtered animals in Italian regions within the first six months of 1992 is 1,245,596 referred to the 12 regions whose data are available. Within 1993, 982 327 animals were slaughtered in regions whose data were available (9/19), Lombardy excluded. During January to April 1994, 153 301 animals were slaughtered in regions whose data were available (7/19), Lombardy excluded. In Lombardy from January to May 1992, 243 025 animals were slaughtered, while in 1993 583 289 animals were slaughtered. All of these animals were submitted to veterinary inspection and a total of 30 outbreaks were detected through inspection of slaughtered animals.

The type of sampling (biological material), the flux of this surveillance actions and laboratory test have already been presented in the previous chapter. Meat inspection, in infected countries strongly contribute to the success of the eradication plan with the following action/result:

(a) Detection of new outbreaks;
(b) monitoring of the disease evolution;
(c) evaluation the efficacy of the eradication measures that have been implemented;
(d) confirmation that the country is again free.

4.2   Condition: the country is carrying out a "vaccination" eradication strategy

When same general situations, i.e. difficult environment, lack of funds, impossibility to control animal movement, communication problems, poor laboratories, etc., hamper the implementation of the "test and slaughter" strategy — the only way to eradicate the disease in a short time is vaccination which remains the only acceptable method to control CBPP. The quality of vaccine, actually used, is under discussion by the scientific community and a new vaccine is expected (CBPP/PARC/EEC project) to cover cattle population of the developing countries.

To survey the trend of the disease and its distribution in the regions, to detect new outbreaks under the above-mentioned conditions, clinical examination and meat inspection represent the unique tools to control the disease. Also in these countries, due to the antibiotic treatment, clinical signs often are very light and sometimes, if veterinarians are not refreshed on CBPP (see Tanzania recent epidemics, TCP/FAO 1995 to 1996), may be confused with other infectious diseases. Meat inspection, as a routine veterinary activity, may allow the detection of new outbreaks in free regions and to evaluate the efficacy of vaccination campaigns (Uganda CTP/FAO 1994). Anywhere animals are slaughtered and organs and meat are sold for human consumption, a veterinarian may detect the disease and take more appropriate and possible actions.

5 Conclusion

Meat inspection is the unique tool for the CBPP epidemiological surveillance that may be used, with good results and low costs, in any sanitary status of a country and under any conditions. Results are linked to the quality of the Veterinary Services, but its contribution to the eradication plans and to maintain the free status is out of discussion.

According to all the above arguments and in order to harmonize the meat inspection CBPP surveillance activity, we suggest that a document/protocol be prepared to indicate to the Veterinary Services all the steps (on technical, statistical and epidemiological basis) which must be followed to ensure the quality of results of this action.

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CONVENTIONAL AND MODERN SEROLOGICAL TECHNIQUES FOR CBPP SURVEILLANCE

F. Thiaucourt

1　Introduction

"Epidemiological surveillance is a method based on continued recording that allows the state of health or the risk factors of a defined population to be assessed." Previous historical examples have shown that eradication of CBPP could be achieved without the help of any laboratory confirmation technique such as serology or any vaccination campaigns. It took four years, from 1890 to 1893, for the United Kingdom to become free of CBPP once the stamping out policy was efficiently put in force. The same policy enabled the United States to become free in 5 years, from 1887 to 1891 (Nocard 1898). More recently, Botswana regained it's freedom status by depopulating completely the cattle population in an area that had been contaminated by transboundary illegal movements.

The success of an eradication campaign mainly depends on the political will and economical abilities of states to enforce unpopular measures that include:

- Slaughter of all animals from infected herds; and
- Restriction of animal movements in infected zones.

At the present time, vaccines do not confer a long lasting immunity nor do they protect 100 percent of the vaccinated animals. As a consequence, vaccination alone can only help in controlling the disease but cannot lead to eradication.

Serological techniques are tools of which the results can be analyzed in order to define the best cost-effective strategies. Decision-makers using the serological results have to be well aware of the characteristics of the tests in order to draw sound conclusions. There are roughly four different serological techniques that can be applied for the detection of antibodies to *M. mycoides* subsp. *mycoides* SC (*Mmm*SC), the CBPP agent: Rapid Slide Agglutination Test (on blood or serum), Complement Fixation Test (CFT), competitive ELISA and Western Blotting. Each of these tests have advantages and drawbacks.

2　Rapid slide agglutination test

This test uses an inactivated whole cell antigen that is coloured (methyl violet). When positive sera are mixed with this antigen, an agglutination occurs.

2.1　*Advantages*
The test is fast: agglutination can be read after two minutes.
It is very simple: there is no need for skilled technicians.
There is no need for apparatus.

2.2　*Drawbacks*

It is quite difficult to perform a great number of analyses at the same time.

The quality of the antigen may vary from one laboratory to another.

The sensitivity of the test is poor; it will detect animals in the early stage of the disease.

The specificity might also be poor and false positives can occur.

2.3 Conclusion

This is a very good field test for the rapid confirmation of outbreaks when performed on a herd basis.

Confirmation can be made on the spot and decisions taken immediately.

It cannot be used as a surveillance tool, particularly in CBPP free countries because of the false positive results and poor sensitivity.

It cannot be used as a surveillance tool in enzootic regions because of lack of sensitivity.

3   Complement fixation test

This test is based on the binding of guinea pig complement on the immune complex that forms when positive sera are incubated with an inactivated *Mmm*SC antigen. The remaining complement is visualized by incubating with an hemolytic system made of sheep red blood cells sensityzed with rabbit anti-sheep RBC.

3.1 Advantages

The test is quite rapid once the complement activity has been estimated (around two hours).

It is very sensitive in the case of an acute outbreak.

Vaccination with T_1 strains do not evoke long lasting sero-conversion, thus enabling CFT being used as confirmation techniques in enzootic zones where vaccination has been performed more than three months previously.

3.2 Drawbacks

Some sera cannot be analyzed due to incompatibility.

It lacks sensitivity and may not detect antibodies when sera are harvested long after an outbreak.

Some false positive may occur with various frequencies.

It is difficult to standardize due to the use of labile biological reagents (complement, sheep red blood cells), hence comparisons between laboratories may be difficult in the case of slight positives.

3.3 Conclusion

This test is a very good test for confirmation of outbreaks when it is used by skilled laboratories.

It can also be used as a surveillance tool in non-affected regions provided that it is very sensitive for the detection of new outbreaks (measurement of incidence).

The occurrence of false positives may shed some doubts on the usefulness of this technique.

4   Competitive ELISA

This test is based on the competition between bovine antibodies and a mouse monoclonal antibody that is specific for *Mmm*SC. The use of a conjugate to mouse immunoglobulins permits to calculate the bound MAb, hence a percentage of competition by comparison with controls.

4.1   Advantages
It is very specific.

Sensitivity is similar to CFT in the case of an acute outbreak; it might be more sensitive in the long term as it detects all bovine IgG subclasses (contrarily to CFT that do not detect IgG2).

Vaccination with T1 strains do not elicit long lasting antibodies.

The test can be fully automated.

Record of data through electronic files and widespread software permit a permanent quality control and statistical analysis.

4.2   Drawbacks
It is a little long to perform if not automated (3 hours 30 minutes).

It requires a well-equipped laboratory.

It might not detect all infected animals at an individual level.

4.3   Conclusion
This test seems to be best suited for large-scale epidemiological enquiries.

In CBPP free regions, it's specificity will decrease the number of false positives that require additional testing or field enquiries.

In enzootic regions it may enable the detection of all infected herds, hence the measurement of CBPP prevalence.

5   Western blotting

This test is based on the migration of denatured proteins in an acrylamide gel according to their molecular weight, and their transfer to a nitrocellulose membrane. Each serum is incubated with a sensitized strip of *Mmm*SC antigen and the bound antibodies are visualized through an immunoenzymatic system leading to dark spots on the nitrocellulose. Sera from CBPP infected animals have antibodies that recognize the immunodominant antigens thus giving a typical pattern.

5.1   Advantages
The test can differentiate a non-specific binding from one resulting from CBPP infection.

5.2   Drawbacks
It is quite cumbersome to perform.

It requires skilled technicians.

It might be difficult to standardize and to use on a large-scale.

5.3   Conclusion

It is a good individual test that can be used for confirmation with sera that have given dubious results with other tests, especially in CBPP free regions.

6 Conclusion

These brief descriptions of ancient and modern serological techniques were not meant to be comprehensive. Their goal was simply to reassess their main advantages and drawbacks in order to foster discussions during the FAO consultative group meeting on CBPP. One of the main points that emerged is that most of these serological tests are very helpful in determining the *CBPP status at the herd level.* This might be the case for any type of serological test that detects, by definition, antibodies. This is certainly more important for CBPP as incubation periods may vary a lot. Hence the unit that has to be taken into consideration for CBPP decisions or regulations is the herd. In that sense, regulations concerning the trade of animals should certainly take into account the herd of origin and not only the traded animals. A further limit of serological test could be the appearance of strains of lower pathogenicity that do not elicit detectable amounts of antibodies, although quite speculative this idea has to be taken into consideration.

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TRANSBOUNDARY ANIMAL DISEASE INFORMATION SYSTEM (TADINFO) FOR CBPP

Roger Paskin

1   Introduction

In fulfilling its responsibilities with regard to transboundary disease management, EMPRES proposes to put in place a global disease monitoring network. This network will enable progress with disease eradication to be monitored, and will assist in highlighting those areas needing extra attention. The network will have a hierarchical structure, with national, regional and global components. Information gathered at a national level will be processed and used nationally, but also fed into a regional component for coordination at the regional level, and then to FAO headquarters at global level.

The information system will entail the development of computer software for the storage and analysis of CBPP (and other disease) data, and an increasingly active role to be played by FAO in strengthening the surveillance capabilities of national veterinary services. FAO is currently engaged in the development of specialized disease data capture and analysis software — TADInfo (Transboundary Animal Disease Information system). This software will have three different modules, designed to function at the national, regional and global levels respectively.

While the focus of the system will be on transboundary animal diseases, the national component of TADInfo is designed in such a way as to be able to capture all disease events which are considered of importance at national level.

2   The information system at national level

TADInfo makes provision for the storage and analysis of data from a number of sources. Visual/clinical surveillance data (which may be active, purposively searching for CBPP, or passive) are catered for in one of the submodules. In addition, provision is made for the capture of data from serosurveys. Know the problems presented by CBPP serology, the software will be able to calculate estimated true prevalence from serosurveillance data, on an area-by-area basis. Of greater importance to CBPP surveillance, particularly as one travels further down the OIE pathway, is the ability to store and analyze abattoir and slaughter slab data. Another submodule of the programme will cater for this.

- Data will be able to be visualized on a simple GIS.

The most complex module, that dealing with data gained from visual surveillance, has been developed as a prototype, and field trials of the software are to begin shortly. This programme is designed to collect detailed data, much of which is relevant to national use only. Not only will the software record data on suspected and confirmed CBPP occurrence, but it will also contain a "search engine" which will identify areas where CBPP-like clinical signs have been seen as a starting point for follow-up.

TADInfo will also be capable of storing data on vaccination campaigns, and, by making comparisons with livestock census data, be able to calculate vaccination coverages. Provision will also be made for queries to ascertain such things as what proportion of reported cases are actually confirmed, the numbers of surveillance reports per unit of cattle population being received from each district, monthly incidence, and so on — in other words, basic epidemiological and managemental tools are incorporated.

It goes without saying that having such powerful software presupposes an efficient field surveillance system and a functional epidemiology unit, all manned by trained and motivated staff. Without people, software is of no value.

- TADInfo at national level will feed data "upward" to the regional epidemiologist by means of e-mail.

It is recognized that many countries have developed their own very effective information systems, and it is intended that provision be made to import data from these systems into the system at regional level. (A successful prototype of a regional surveillance system has been developed by the SADC countries, whereby information from various national surveillance systems is fed into a regional SADC reporting system.)

3   The information system at a regional level

A separate TADInfo module (with different capabilities from the one operating at national level) is to be developed which will receive data electronically from participating countries. This module may be situated in subregional FAO offices, or reside with other collaborating organizations, e.g. OAU/IBAR, SADC.

Once again, the human resource component of the system at regional level is of prime importance. The main human resource at play here is the regional epidemiologist, who will play a major role in improving surveillance and reporting in participating countries; providing in–country training; organizing regional workshops; publication of disease reports as a feedback to participating countries; verification of data with national authorities; coordinating regional responses to disease emergencies; assisting with the drawing up of contingency plans, both at national and regional level; reporting to EMPRES. A key factor here will be the monitoring of progress with regional control/eradication strategies, and offering of assistance where necessary.

This epidemiologist will be backed up by TADInfo Regional, the software package intended for regional data capture. This software will be linked to a regional GIS so that regional CBPP patterns can be visualised. It will also be possible to visualize progress with vaccination, the results of post–vaccination serosurveys, and to follow monthly disease incidence on a country-by-country basis. This module will also incorporate a capability for quality monitoring of the national systems through agreed performance indicators.

4   The information system at global level

At FAO HQs, data will be received electronically from regional reporting systems. From here, it will be possible to follow global CBPP trends. Apart from being able to visualize the disease occurrence in a GIS, TADInfo global will make provision for the incorporation of geographic disease information as a GIS layer with other layers relating to weather, vegetation, and cattle population density in order to provide elementary risk mapping and disease prediction which would be useful at regional and national level. Development of this type of software is an extremely complex issue, and the ability to automate risk mapping will not be developed for some time, although "manual risk mapping" is possible at the moment. Prior work will be needed to identify and in part, quantify, the promoting and retarding factors for CBPP propagation so that these can be incorporated into the software.

- The idea is that TADInfo should not be part of an academic exercise, but of practical application in terms of early warning for CBPP.

At FAO HQ, data will be received electronically from regional reporting systems. From here, it will be possible to follow progress in Rinderpest eradication worldwide. Apart from being able to visualize the disease occurrence in a GIS, TADInfo global will make provision for the incorporation of geographic disease information as a GIS layer with other layers relating to weather, vegetation, and cattle population density in order to provide elementary risk mapping and disease predication which would be useful at regional and national level. Development of this type of software is an extremely complex issue, and the ability to automate risk mapping will not be developed for some time, although manual risk mapping will be undertaken in the meantime.

5   Conclusion

Putting a global CBPP monitoring system in place presents a powerful challenge to EMPRES, and to CBPP-affected member countries. The success of such a system requires:

- Effective ground-level CBPP surveillance in all affected countries, with personnel effectively deployed, livestock inspection programmes, serosurveillance campaigns and slaughter slab monitoring;
- A willingness to report transparently on all suspected outbreaks on a regular basis and have such outbreaks investigated;
- The existence of computerized disease reporting systems in all affected countries;
- The existence, and regular use of, robust electronic mail connections between member countries and regional offices;
- The existence of regional bodies (FAO or other), manned by competent epidemiologists, with good electronic mail connections to FAO HQ;
- The political will of FAO member countries to actively participate in such a system.

Strong commitment is required from all players in the field. Demands will be made upon EMPRES resources, especially in terms of training and follow-up work at field level. CBPP is, given its spread by transhumant movement, its notorious detection difficulties and its elusive carrier state, one of the world's most troublesome livestock diseases. A computer system alone will not eradicate it, but an international team of dedicated and competent people — from vaccinators to veterinarians, will, with the aid of an effective information system, be well equipped to send CBPP into the pages of history.

Basic conceptual outline of TADInfo
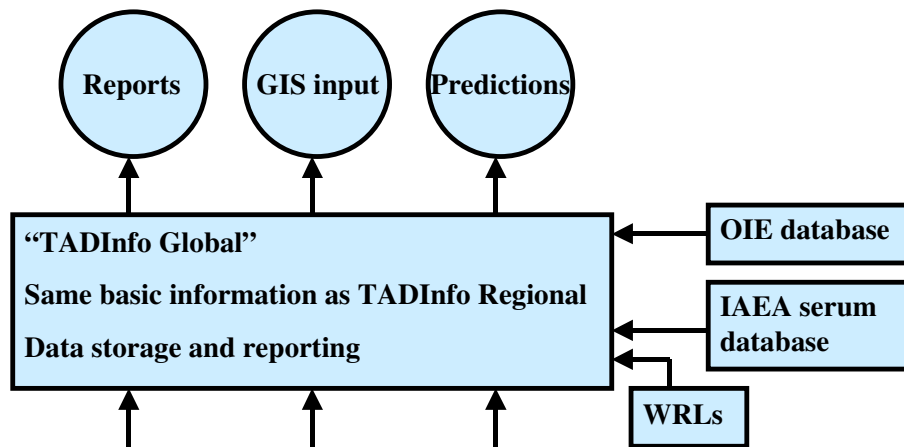
CAUSATIVE AGENT: TAXONOMY AND MOLECULAR EPIDEMIOLOGY

J. B. March

1   Taxonomy of the causative agent

Contagious bovine pleuropneumonia (CBPP) is caused by infection with *Mycoplasma mycoides* subsp. *mycoides* small colony biotype (or *Mmm*SC). This mycoplasma is a member of the so-called 'mycoides cluster', a taxonomic grouping of six closely related mycoplasmas which are all pathogenic to some degree in ruminants (see Egwu *et al.,* 1996 for review). *Mmm*SC has also been isolated from goats and sheep, and as such, *these small ruminants must be considered potential reservoirs of infection* (although no records of transmission to cattle have been reported). It is not currently known if goats or sheep carrying *Mmm*SC can infect cattle. Interestingly, *Mmm*SC was the first mycoplasma to be isolated, by Nocard and Roux in 1898, partly due to the relative ease of culture of this organism. This contrasts markedly with *M. capricolum* subsp. *capri-pneumoniae*, the causative agent of contagious caprine pleuropneumonia (CCPP), and also a member of the cluster. The fastidious growth requirements of this mycoplasma allowed it to escape detection until 1976 (MacOwan and Minette). *One can speculate that perhaps additional members of the 'cluster' await discovery once appropriate media and specific tests become available.* *M. mycoides* subsp. *capri* is also isolated from goats, where it can cause pleuropneumonia, arthritis and mastitis, although the exact pathology is unclear. *M. mycoides* subsp, *mycoides* LC (large colony biotype) causes a pleuropneumonia which is pathologically similar to that seen in CCPP. *M. capricolum* subsp. *capricolum* is also pathogenic for goats (which are the main host) and is mainly isolated from cases of arthritis, although it has shown to cause pleuropneumonia in experimental infections. Finally, *Mycoplasma sp.* Bovine serogroup 7, which causes arthritis in cattle and is serologically related to the agent of CCPP (Belton *et al.,* 1994), although there is little evidence to support its presence in sheep or goats.

Whilst each member of the cluster would appear to have a 'preferred' host animal, evidence is accumulating that mycoplasmas may infact have a broader host range than originally considered. This is particularly relevant considering that a mixed culture containing two or more members of the 'mycoides cluster' may be isolated from an infected animal, making identification of the exact role of each in the disease condition difficult. The high degree of serological and DNA-relatedness between members has hitherto contributed to the difficulty of identification, although the availability of specific tests based upon PCR and monoclonal antibodies (MAbs) should help considerably in this respect.

2   Molecular epidemiology of *M. mycoides* subsp. *mycoides* SC

Although *Mmm*SC is reasonably well classified within the 'mycoides cluster', with both specific serological and PCR–based tests available to distinguish it from other members of the group, molecular epidemiological studies of within-species isolates are not particularly well developed. This is especially so with respect to recent African field isolates, and perhaps reflects a concentration of study into recent European outbreaks instead. *A more detailed epidemiological study of recent African field isolates would be a considerable asset in providing data on the source and spread of recent infections, particularly with regard to the evolution of MmmSC field isolates.* Studies to date are consistent with there being two

main subtypes of *Mmm*SC; those isolated from recent European outbreaks of CBPP (post-1980, of which a considerable number are available), and the African/Australian subtype, many of which are of a much older vintage. European isolates are generally regarded as being of lower virulence than African/Australian strains (although the latter are sometimes attenuated in individual cases by multiple passage). The basis for this apparent difference is not presently understood (see below); whether due to an intrinsic property of the mycoplasma or the result of possibly better husbandry and sanitary conditions under which European cattle are kept. Whether these differences are sufficiently great to allow a formal classification of *Mmm*SC isolates into two subspecies remains a matter of conjecture at present. Unfortunately, all studies described below lack comparison with pre-1980 European isolates (none appear to be available).

2.1 DNA polymorphisms

(a) Insertion sequence analysis. Cheng *et al.* (1995), have used IS*1296* analysis as a means of typing some 64 strains of *Mmm*SC from a wide variety of geographical locations. Results have revealed two main clusters; one containing contemporary European strains, and the other containing Australian and African isolates dating back to 1931. While the latter cluster demonstrated some degree of heterogeneity (as might be expected considering the wide range of geographical and temporal variation), the former group was very homogeneous, with 38 out of 39 European isolates belonging to a single isotype. (b) RFLP (Restriction fragment length polymorphism) analysis by Poumarat and Solsona (1995) provided similar results to the IS*1296* analysis; namely homogeneous groupings of all European strains into a single isotype, with African strains forming a separate, more heterogeneous group. Australian strains were not examined in this study, so their classification into European or African subgroups cannot be made on the basis of RFLP.

2.2 Biochemical diversity

Research in this area (Abu-Groun *et al.,* 1994; Houshaymi *et al.,* 1998) has revealed non-systematic differences between certain strains on the basis of substrate utilization (e.g. glucosamine, mannose and maltose) which may offer potential for classifying isolates from the current African outbreaks. A notable exception however is the systematic difference between African/Australian and European strains with respect to the ability of the former group to utilize glycerol as a carbon source. A by-product of this metabolism is hydrogen peroxide, a known virulence factor during mycoplasmal infections. Whilst this provides a convenient explanation for the increased virulence of African/Australian strains compared to European strains, the whole picture is undoubtedly more complex. There is no evidence that mycoplasmas use glycerol as a metabolite in vivo, and furthermore, some African isolates (e.g. vaccine strain KH3J) can utilize glycerol and yet are unequivocally of low virulence (see below). Unfortunately, biochemical tests are relatively complicated and require specialized equipment, thus limiting this area of research and its application to field studies.

2.3 Capsular polysaccharides (CPS)

To date, the extracellular capsule of *Mmm*SC is the only virulence factor shown to be important *in vivo*. Studies (March *et al.,* 1998) have demonstrated differences in the amount of CPS produced by strains (see Appendix I), and an effect on virulence is known (e.g. low virulence vaccine strain KH3J becomes much more virulent if supplemented with additional

CPS during challenge (Hudson *et al.,* 1967). However, the stability of the trait is unknown, and accurate measurement of CPS is not an easy undertaking. Typing of strains using CPS-specific MAbs may however offer potential due to the low level of cross-reactivity observed with other members of the 'mycoides cluster'. Work is underway to determine whether European and African/Australian strains of *Mmm*SC also demonstrate subgrouping on the basis of CPS yield or type.

2.4 Serology

A substantial bank of MAbs against *Mmm*SC currently exist (e.g. Le Goff and Thiaucourt 1998), which may have considerable potential in strain typing. Their use to date has been mostly restricted to diagnosis, in which the ability to recognize all strains of *Mmm*SC without unwanted cross–reactivities has been the overriding concern. *MmmSC MAbs might offer considerable potential for serotyping of current African isolates*, particularly since the specificities and target proteins of each MAb can be easily determined. The work involved in such an analysis would not be excessive, particularly if shared between collaborating laboratories (e.g. one laboratory might be responsible for antigen production whilst another could undertake the serology).

2.5 Protein banding

Research by Poumarat and Solsona (1995) comparing the antigenic profiles of 46 strains of *Mmm*SC resulted in seven different patterns spread across the isolates from Europe and Africa. Polymorphisms were observed in five different 'zones' (i.e. protein molecular weights), with European strains forming four isotypes and African strains the remaining three. Apart from African and European strains never being present in the same isotype, it is difficult to draw specific inferences from these studies. Studies by Gonçalves *et al.* (1994, 1998), have provided similar data; a large number of polymorphisms of specific protein bands, but no real pattern with respect to particular isolates. These latter authors did, however, report on the presence of a 72/70 kDa band present in African/Australian isolates but absent in European strains, in agreement with the premise of reproducible differences between these 'subgroups' remarked on above. Although these studies do observe a few specific polymorphisms between strains (e.g. similarities in banding patterns between Italian and Portuguese isolates suggesting a common source of infection), taken in isolation it is difficult to assess the significance of these observations.

3 Conclusions

Although clear distinctions can be made between European and African/Australian isolates of *Mmm*SC on the basis of numerous criteria (PFLP, IS*1296*, protein banding), it is difficult to draw conclusions on the relatedness or evolutionary history of strains within these subgroups, particularly since data is often presented in isolation with no reference to location or time of isolation. Studies have concentrated heavily upon recent European outbreaks of CBPP; unfortunately, little comparable data is available to allow an assessment of the source and spread of the current African epidemic. Several authors have already commented upon the conundrum that CBPP in Africa/Australia was introduced from Europe (at least in several well-documented historical cases), and yet *Mmm*SC strains from these locations bear far more resemblance to each other than to current European isolates. If CBPP already pre-existed in Africa before the introduction of European strains than these findings are even

more perplexing — how can the descendants of introduced strains have evolved to bear more resemblence to pre-existing stock than their ancestors? The high level of homogeneity currently observed with European strains would, if anything, tend to suggest that their rate of evolution is not high. The only logical explanation of these findings would appear to be that African/Australian strains are (at least in part) descended from a different (more virulent?) European ancestor than is currently found on the continent. The assumption being that the virulent European strains responsible for outbreaks in the nineteenth and early twentieth centuries have been eradicated by a policy of slaughter. Presumably, low virulence allows current *Mmm*SC strains to persist in low-level chronic infections in Europe.

Finally, two important features of the above data on molecular epidemiology have to be considered. *Firstly, little is known about the rate or type of evolution of MmmSC following laboratory culture. It is possible that observed polymorphisms may represent an adaptation to culture in vitro rather than reflect the true phenotypic situation of freshly isolated strains.* This may be particularly relevant when comparing multiply-passaged strains such as PG1 with recent field isolates which may have only been subcultured on a few occasions. Finally, whether these observed polymorphisms actually represent functional alterations in virulence and/or isotyping, or whether they simply represent irrelevant genetic changes remains to be determined in most cases.

Appendix I: Current African outlook

Only very sketchy information is available on the molecular epidemiology of current African isolates of *Mmm*SC. A more detailed study here would undoubtedly be of great benefit in tracking the spread of routes of infection, and provide data on the rate of revolutionary change, particularly with respect to protective epitopes and/or virulence factors. Early data on the appearance of new isotypes would be of considerable use in assessing the likely impact of vaccination strategies. Studies in our laboratories and others (March *et al.,* 1998; Houshaymi *et al.,* 1998) have suggested that at least two isotypes of *Mmm*SC are involved in the recent outbreak of CBPP in Botswana (Amanfu *et al.,* 1998), on the basis of (1) strain morphology, (2) CPS yield, (3) antigenic profiling, and (4) biochemical differences. The presence of multi-factor differences in strain identification allows greater confidence in such an assessment. Strain M375 was isolated at Mohenbo, close to the Angolan border in November 1995, while strain N6 was isolated at Nokaneng, further south, almost a year later. These differences may indicate separate Angolan and Namibian sources for the two strains, although, interestingly, two contemporary Tanzanian isolates are indistinguishable from N6 using the same criteria. However, it is difficult to propose a route by which geographically separate locations such as Tanzania and Botswana could have acquired a common source of infection. Two interesting speculations can be made; firstly, from the tests we conducted, we could not distinguish any differences between strain N6 (or the Tanzanian isolates) and the vaccine strain T_1SR. *Possibly poor vaccination technique might have led to a vaccine strain to revert to virulence?* Secondly, strain M375 is clearly different from all other African and vaccine strains examined. Perhaps the strain might have a contemporary European origin? Clearly, a more detailed study, comparing representative strains of each subgroup and a variety of molecular markers would be of considerable use in assessing this. *A central laboratory, perhaps to which representative samples from current outbreaks could be sent and in which a series of standardized molecular studies could be done would be of considerable benefit.* Many of the tests are not especially difficult or expensive to perform

(e.g. RFLP, IS analysis, antigenic profiling), and if performed under standardized operating conditions should yield highly reproducible and significant data. *An alternative strategy may be for several laboratories to collaborate in an Africa-wide survey, each with a specific role (e.g. RFLP, antigenic profiling) depending upon expertise. European Union or camparable funds may be accessible to enable this type of cooperative project to be set up.*

Appendix II: PG1 as the type strain of *Mmm*SC — the need for reappraisal?

PG1 is the international reference strain for *Mmm*SC (Edward and Freund, 1973); however, molecular epidemiological studies suggest that it is perhaps atypical of the species. The geographical origin of the strain is not known, nor the tissue from which it was isolated. Furthermore, nothing is known about the passage level of the strain nor the original pathology of the animal from which it was isolated. Antigenic profiling studies by several authors (Poumarat and Solsona, 1995; Gonçalves *et al.,* 1998), have suggested that while PG1 clearly fits into the African/Australian subgroup, it displays a unique pattern of polymorphisms which sets it apart from the other strains tested. Again, IS*1296* analysis (Cheng *et al.,* 1995) has suggested that PG1 is of the African/Australian grouping, but that it exhibits a unique pattern. Unpublished work has also suggested unique polymorphisms for this strain: 16S ribosomal RNA sequencing suggests that while most strains are extremely homogeneous, PG1 displays a unique polymorphism in the sequence (Anja Persson, personal communication). While most strains can be differentiated from each other on the basis of a single difference (whether it be RFLP, IS*1296*, antigenic profiling etc.), PG1 is apparently unique in that in practically every category it can be differentiated from other strains. This may be a reflection on the evolution of field strains in the relatively lengthy period since 1931 when PG1 was first described, or it may simply be a case of an unlucky choice of which strain to designate type strain at a time when the above tests were not available to allow these taxonomic studies to take place.

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# CAUSATIVE AGENT: DIAGNOSTIC TECHNIQUES

R. A. J. Nicholas

1   Introduction

The isolation and growth of *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC), the causative agent of CBPP, is essential for the the confirmation of outbreaks particularly in new areas. It is also a requirement of the OIE for countries wishing to declare freedom from CBPP under the recommended standards for epidemiological surveillance systems for the disease (1997).

The organism is not intrinsically difficult to grow unlike many other fastidious mycoplasmas such as that causing CCPP but does require a fully functioning bacteriological laboratory with aseptic facilities for preparing a high quality growth media. Figure 1 shows the classical pathway to achieving the isolation, culture and identification of the *Mmm*SC.

Figure 1.

Sample collection
↓
Transport
↓
Growth
↓
Cloning
↓
Biochemical tests
↓
Serological identification
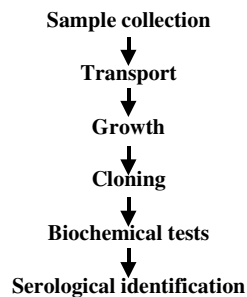
2   Sample collection

It is widely known that in the living animal nasal secretions, nasal swabs, tracheal and broncho-alveolar washings, and even pleural fluid may yield *Mmm*SC, though more successfully from acutely affected animals; in exceptional circumstances the mycoplasma may be found in the urine, blood and synovial fluid. In the dead animal, pleural fluid, congested lungs and the inner capsule of sequestra, associated lymph nodes and kidneys with lesions are all recommended sites.

3   Transport of samples

The rapid and cool transport of the samples to the laboratory in a suitable media is a crucial and often overlooked factor in the success of mycoplasma recovery particularly in hot climates. A balance must be struck between ensuring the survival of *Mmm*SC and reducing contamination by other bacteria. Thallium acetate and a range of antibiotics have been used widely to suppress cell walled bacteria although these too will be overwhelmed by gross contamination. The addition of selective inhibitors like nisin and amino acids can control effectively acholeplasmas and arginine hydrolysing bacteria which are often isolated from

cases of CBPP occasionally at the expense of *Mmm*SC (Ozcan and Miles 1996; Abu-Amero *et al.,* 1996).

4 Growth of mycoplasmas

Many media have been described to support the growth of *Mmm*SC and where the mycoplasmas are abundant few problems should be met. However, where infection is slight or in antibiotic treated animals, recovery will be more difficult. The essential ingredients are: a protein digest, fresh and dried yeast extract, serum, glucose, buffer and mineral salts; aeration is also thought to be desirable. Under the EC FAIR project on CBPP a medium called PRM has been developed which gives a maximum yield with 48 hours and is particularly suitable for antigen and vaccine production (Miles, unpublished report). A commercial medium (Mycoplasma Experience, UK) is also available for laboratories which cannot produce their own.

5 Cloning

Cloning is necessary to prevent the overgrowth of *Mmm*SC by other mycoplasmas and acholeplasmas. However, it can delay the diagnosis of CBPP considerably. Attempts should therefore be made, in parallel to cloning, to achieve identification directly on the recovered isolates.

6 Biochemical tests

There are no biochemical tests which will provide definitive diagnosis for *Mmm*SC. Instead they may be used to differentiate closely related mycoplasmas such as those belonging to the *M. mycoides* cluster. A few tests are useful such as casein digestion which distinguishes *Mmm*SC from *Mmm*LC (Nicholas and Bashiruddin, 1995); others are subjective and do not apply to all strains. Furthermore, Houshaymi *et al.,* (1997) recently showed that European strains of *Mmm*SC differed from African and Australian strains in their inability to hydrolyse glycerol.

7 Serological identification

Growth inhibition and immunofluorescence tests are widely used and apart from some minor cross-reactions with closely related mycoplasmas are generally satisfactory. Metabolic inhibition tests have the advantage that they do not require mycoplasma growth on solid media.

8 Polymerase chain reaction tests

The introduction of PCR to the detection and identification of *Mmm*SC has many advantages over traditional techniques:

- The test is rapid and specific (several hours);
- the test can be carried out on mixed cultures early in the isolation procedure;
- no cloning is necessary;
- detection may be directly from clinical material particularly pleural fluid.

In samples where *Mmm*SC is abundant, no DNA extraction is necessary. The disadvantages are that the test is expensive to set up and requires highly trained personnel. Many PCRs have been developed: that based on the CAP–21 sequence is robust, but requires a restriction enzyme step to differentiate *Mmm*SC from *Mmm*LC (Bashiruddin *et al.,* 1994); a related PCR also enables the detection of all members of the *M. mycoides* cluster. Others include specific PCRs for *Mmm*SC (Dedieu *et al.,* 1994; Miserez *et al.,* 1997).

9   Immunocytochemistry

This test together with the immunofluorescent test should not be overlooked when setting up diagnostic tests for CBPP. Tissues from the abattoir or from the field can be conveniently preserved in formalin before sectioning and testing in the laboratory. The availability of monoclonal antibodies has significantly improved specificity and reduced background satining (Ayling 1998a). As for sensitivity, ICC compares very favourably with cultural isolation because of the practical problems of sample handling and transport.

10   Other antigenic detection systems

The major challenge for CBPP diagnosis is the development of robust penside tests which would enable immediate diagnosis and control particularly in remote areas and with moving cattle populations. This approach is further advanced for antibody detection with rapid slide tests and more recently latex agglutination tests (Ayling *et al.,* 1998b). The use of antigen detection ELISAs or latex beads coated with specific monoclonal antibodies may be better suited to African laboratories. However, those reported so far require pre-enrichment stages which limit their usefulness (Rodrigues *et al.,* 1996). Paffard *et al.,* (1997) have recently reported an ELISA test which used antibody impregnated membranes to capture *Mmm*SC after an immunomagnetic separation of the mycoplasma from clinical material which can be completed in one hour.

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CAUSATIVE AGENT: *M. MYCOIDES* SUBSP. *MYCOIDES* GENOME, VIRULENCE AND IMMUNOGENICITY

J. B. Bashiruddin

1   Introduction

The genome of *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC) is probably responsible for the orchestration of products which give the organism virulence and immunogenicity. Many gene products may act as virulence factors such as adhesins, mitogens, and extracellular compounds.

*Mmm*SC like others in the same Genus, have small circular genomes with low guanine and cytosine (G+C) contents. The size of the genome of *Mmm*SC, type strain PG1, has been estimated at 760 kbp or 810 kbp or 923 kbp or 1 280 kbp depending on the experimental technique, but the latter, estimated by pulse field gel electrophoresis, is on the higher side in the range 580 to 1 400 kbp for mycoplasmas. The amount of G+C of 26 Mol percent is more or less average for these organisms. Early studies of the whole genomes of members of the *M. mycoides* cluster by DNA-DNA hybridization found 90 percent homology between *Mmm*SC, strain PG1 and *M. mycoides* subsp. *mycoides* LC (*Mmm*LC), strain Y goat. This work which grouped *Mmm*SC and *Mmm*LC together separated *M. mycoides* from the other organisms in the *M. mycoides* cluster. Later the technique of gene mapping showed differences in size and organization of the genome of these closely related organisms.

Structural maps of genomes may be constructed by multiple restriction enzyme digestion of the extracted DNA and the patterns observed form the basis of genetic maps. Genomic maps thus constructed have shown that *Mmm*SC forms a group separated from *Mmm*LC. The *Mmm*SC strains analyzed were found to be more homogeneous than *Mmm*LC in these studies. Probes from cloned fragments of other mycoplasmas were used to locate genes on the genomic maps by hybridization. The relative locations of a few genes for transfer RNA, ribosomal RNAs, and some enzymes were identified on the *Mmm*SC genome. Two peculiarities of the mycoplasmal genome hamper the identification of mycoplasma genes by these methods. The first is that their low G+C content offers poor complement for cloned, defined probes which are usually derived from other bacteria. The second is the altered codon usage of mycoplasma in which UGA codes for tryptophan instead of the termination signal making full length mycoplasmal genes difficult to clone in bacteria.

Some parts of the *Mmm*SC genome are quite well known for example the 16S rRNA and the integration sequence, IS*1296*. Genetic analyses of these areas of the genome have shown not only similarities, but also differences between *Mmm*SC strains.

2   Similarities between *Mmm*SC strains

At about the same time as the compilation of genomic maps of *Mmm*SC strains, southern hybridization studies with a *M. mycoides* cluster-specific gene probe prepared from a cloned fragment of *M. mycoides* subsp. *capri* genome, were being experimented. This work found a very close relationship between the homologous organism and *Mmm*LC, and a difference between the *Mmm*SC and *Mmm*LC. It also showed that *Mmm*SC strains isolated from a

variety of geographical areas and from large and small ruminants were very similar. A *Mmm*SC-specific probe, developed from another part of the genome, was also able to detect a wide range of isolates. Both of these studies led to the development of diagnostic tests and more sensitive polymerase chain reaction (PCR)-based tests for diagnosis of CBPP; the specificity of the reactions could be confirmed by restriction enzyme digestion of the amplification products. The high sequence homology between strains was evident in both the regions of DNA because all strains and isolates tested produced PCR products of the expected size and the restriction enzyme sites were consistent within these fragments.

A DNA fragment containing the genes for the immunoreactive protein, p72, and IS*1296* was cloned and sequenced. Similar sequences to IS*1296* were found in *Mmm*LC and bovine group 7 mycoplasmas, and related sequences to the p72 gene are probably present in *M. capricolum* subsp. *capricolum* and bovine group 7 mycoplasmas. Nevertheless, a very sensitive nested PCR system has been developed from these sequences which is capable of specific detection of *Mmm*SC.

Recently, the sequences of the genes coding for 16S rRNA have been analyzed. Members of the *M. mycoides* cluster have two operons for rRNA, designated *rrnA* and *rrnB*. Operon *rrnB* of *Mmm*SC has 7 polymorphisms or sequence differences from the other operon which is low compared with some other mycoplasmas tested, for example 11 to 17 polymorphic sites in *M. capricolum* subsp. *capripneumoniae* strains. One of these polymorphic sites is in a restriction enzyme site and this was used to produce a specific diagnostic PCR test which correctly identifies all *Mmm*SC isolates. Another polymorphism in the 16S rRNA genes was found in the sequence length between the two operons around the nucleotide positions 1264 to 1270. The *rrnA* operon contained 7 adenosines in this region, whereas the *rrnB* operon only contained 5 adenosines. Other members of the *M. mycoides* cluster do not display this variation and have 6 adenosines at similar positions in both the operons. A PCR test was designed to detect this difference in length and the double band thus produced is a characteristic signature of *Mmm*SC.

3   Differences between *Mmm*SC strains

Some very powerful detection and identification systems have utilized and rely on the similarity of *Mmm*SC genomes. However, comparison of the genomic maps of several *Mmm*SC strains show small differences in total sizes and distances between some restriction sites. Restriction enzyme analysis of 10 African and 15 European strains indicated major differences between these two groups implying that the source of recent outbreaks in Europe was not of African origin. Although no discriminatory polymorphism was identified in European strains of *Mmm*SC, PG1 and one Tanzanian isolate had an extra polymorphism each of which were in different positions.

The IS*1296* occurs in 18 to 20 copies only in *Mmm*SC and this allowed differentiation between strains of *Mmm*SC by the DNA fingerprinting technique. Ten discernible patterns were evident and the matching of these grouped African and Australian strains together while European strains, which were quite stable in themselves, formed another distinct group. Significantly, this technique permitted the differentiation between strains and may be able to consistently distinguish vaccine strains from others.

4   Virulence and immunogenicity

The occurrence of *Mmm*SC strains of differing virulence have been reported. Naturally-occurring isolates with reduced virulence, and adapted strains with reduced pathogenicity have been used as vaccines against CBPP. Evidence of altered virulence has been heard on several occasions. Freshly-isolated strains lose their virulence on extended passage. Strains isolated early in an outbreak appear more virulent that those isolated later from the same outbreak. Recently, cattle from the first outbreaks in Botswana presented with severe clinical symptoms of CBPP unlike those towards the end.

Galactan has long been suspected as one of the virulence factors of *Mmm*SC. It was found to be a component of a diffusable toxin capable of causing necrosis by itself and induced a tissue response which formed structures similar to sequestra of chronically infected animals. Virulence has been directly related to the production of galactan and strains producing less of these substances have been shown to be less virulent and mildly pathogenic and more easily cleared by natural defence mechanisms. In itself, galactan is not immunogenic, but after injection it produced anaphylactic reactions when the host was challenged with the organism.

Adhesins have been detected in a number of mycoplasmas organized into tip structures. The P1 protein of *M. pneumoniae* is the major factor but not the only protein involved in cytadherence. Genes for these proteins are clustered in 4 groups around the genome. Similar genes in *M. genitalium* are all clustered together, and this suggests that their regulation is concerted. In *E. coli* clusters which code for virulence factors are known as pathogenicity islands. Many have the ability to rearrange themselves and are associated with transposable elements such as insertion sequences. A P1-like protein has not been described in *Mmm*SC.

A haemolysin has been mentioned and some workers have sequenced part of this putative gene. But it is known that *Mmm*SC produce peroxide which in itself is haemolytic. This is the result of a flavin-terminated electron transport chain (ETC). Recycling of the end product results in the production of a small amount of peroxide. So the addition of glucose stimulates the production of peroxide. Recently it was shown that much more peroxide was produced from glycerol by African and Australian *Mmm*SC strains. In other organisms such as *E. coli*, glycerol is actively transported into the cell, converted to glycerol–3–phosphate which is then converted to dihydroxacetone phosphate by a NAD dependant dehydrogenase. If this reaction was performed by an enzyme which used oxygen, the reaction would produce peroxide at levels higher than that produced as a result of glucose catabolism. Thus, it is proposed that an enzyme, glycerol–3–phosphate oxidase, which uses oxygen directly is responsible for peroxide production but it has not been described in *Mmm*SC.

Peroxide production may be a general feature of mycoplasmas as catalase helps survival of mycoplasmas in culture. No catalases or peroxidases have been described for *Mmm*SC, and it has been proposed that mycoplasmas may kill themselves by the production of their own peroxide. Maybe they use the hosts mechanisms to clear themselves of this toxic product.

An increased production of hydrogen peroxide by African and Australian strains of *Mmm*SC during glycerol oxidation *in vitro* led to speculation concerning the role of oxidative damage in pathogenesis. It is supposed that oxidative damage is caused by a combination of mycoplasmal and unchecked host systems. *Mmm*SC has to protect itself against oxidative

damage on the surface and these surface structures will be exposed to the host immune systems, and be immunogenic. The surface proteins of *Mmm*SC are immunogenic, and proteins p110, p98, p95, p62 and p48 and maybe p37 of *Mmm*SC are among these.

Some mycoplasmas have mechnisms to change their surface proteins with a high frequency. *M. bovis* has 3 variable surface proteins (vsp) which are membrane anchored, hydrophilic polypeptides. The genes which code for these are clustered together and undergo phase variation, that is, their expression may be switched on and off. They also are capable of variable co-expression which results in mixtures of these proteins on the surface of cells. The genes are also able to vary their size which results in size variation of the proteins expressed. A similar system of *M. hyorhinis* has an IS associated with it. There is some evidence that two membrane-associated proteins of *Mmm*SC may belong to this group.

Host factors and age also modify pathogenicity. The susceptibility to CBPP increases with age of the animal. Calves under six months of age which develop only minor lesions of tendons and joints and seldom pulmonary lesions whereas many organs including the lung, kidneys, and brain, of older animals may be affected. The disease in these animals is more severe. Symptoms and immune response may be modified by antibiotics.

5   Concluding remarks

*Mmm*SC, compared to some other members of the cluster, are a homogeneous group of organisms. Parts of the genome are stable and other parts are variable. The basic mechanisms of virulence are probably the same for all strains, but immunogenicity (and pathogenicity) may also be modified by the host. These virulence functions of *Mmm*SC have been difficult to decipher. Genetic studies are now becoming as sophisticated as those for bacteria, and toxins, adhesins, and immunoreactive molecules are being described. Many functions of the genome and the intimate interactions between the host and *Mmm*SC remain to be discovered.

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CAUSATIVE AGENT: CBPP PATHOGENESIS

A. Provost

1   Accepted facts (reminder)

1.1   Anatomy
- Continuous production of lymph in the alveoles;
- this lymph migrates through broncho-alveolar slots, then into epithelium-like bordered conducts, then into structured lymphatic ducts;
- these ducts form muff-like structures around intra-lobular arterioles and venules;
- pleural and perilobular spaces are interconnected at the level of the lobar pediculum, not at the hilum level.

1.2   Immunology
- Antigenic (close) relationship between *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC) galactan and a bovine lung pneumogalactan;
- intradermal (= intralymphatic) inoculation of *Mmm*SC ensues in a transient mycoplasmaemia, without lymph node block;
- in natural disease, the rise of antibodies is late (several weeks) after infection; first detectable antibodies are precipitins. Their apparition is comtemporary with, or preceeding, a rise in temperature and first objective signs of lung involvement;
- in other words, serological conversion appears 2 to 5 days before the clinical signs. The build-up of lesions is rapid;
- a hypersensitivity stage occurs during *Mmm*SC infection. It is detectable by intradermal infection of *Mmm*SC polyosidic extracts. A genuine Arthus phenomenon develops, i.e. capillary thrombosis surrounded by PMN cell layers.

An Arthus phenomenon develops where there is a right balance between an allergen (= antigen) and its corresponding precipitating antibody; the precipitating antibody should have a high titre in order to produce *in veino* an immuno-complex facing the antigenic extravascular depot.

1.3   Clinical aspects
- Long incubation period, much longer than in any respiratory disease;
- abrupt occurrence of clinical signs; after serological rise;
- acute disease of short duration;
- in an outbreak, less than half of infected cattle have an hyperacute or acute form;
- at the time of contamination, short transient fever ("fièvre périmonitoire de Laquerrière").

1.4   Pathology
- Pleural exudation and/or serious infiltration of perilobular spaces are previous to any oedematous involvement of the lobules; but is contemporary with an inflammatory and oedematous involvement of the mediastinal lymph nodes;
- the occurrence of PNM in lymphoid spaces is rather surprising;
- the involvement of the pulmonary alveoles in the process is subsequent to the perilobular reflection;

- adjacent lobules are at different stages of evolution, and, in an affected lung, circumscribed lesions may be found at different stages of evolution.

1.5 *Epizootiology*
- *Mmm*SC is present in bronchial and nasal mucus in diseased animals;
- cough gives rise to 1 to 3 µm droplets; only the 1 µm size droplets are able to reach the alveoles;
- direct transmission prevails.

VACCINES AND VACCINATION: VACCINE QUALITY ISSUES IN AFRICA, INCLUDING SAFETY AND EFFICACY

Joseph K. Litamoi and B. M. Seck

1   Introduction

Contagious bovine pleuropneumonia (CBPP) is an economically important disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC).   On account of its infectiousness, the potential for its rapid transboundary spread and the associated economic impacts, CBPP is now amongst the OIE list A diseases.   For similar reasons CBPP is included in the list of 6 priority diseases for FAO's EMPRES–Livestock programme.   In addition the second priority vaccine for the Pan African Veterinary Vaccine Centre (PANVAC) is that for CBPP (1, 2, 3).

Over time the control and eradication of CBPP has been carried out using a variety of methods.   The most successful procedure has been the stamping out policy of slaughter of infected and/or exposed cattle along with other zoo-sanitary measures including strict control of animal movements. A combination of cattle movement control, slaughter and vaccination were effective in eradicating CBPP from Australia (4). For a variety of factors (including socio-economic, geographic and husbandry practices) the methods of slaughter (with compensation) and strict cattle movement control may not be feasible in most of the African continent at present. Consequently, the most realistic options remain mass vaccination and where possible, animal movement control (4). To be effective a programme for CBPP control through vaccination must ensure high immunization coverage using good quality vaccines which should be repeatedly administered at short intervals.

For this reason the Pan African Rinderpest Campaign (PARC) of OAU/IBAR which also coordinates CBPP control programmes in Africa now requires that the CBPP vaccines to be used in their disease control undertakings must have been tested and certified by PANVAC to be of good quality.

The objectives of the PANVAC CBPP vaccine quality control activities which commenced in 1991 are to assist producer laboratories in Africa to manufacture vaccines in conformity with the OIE requirements for such products.   This presentation reviews the results of the PANVAC CBPP vaccine quality testing since its inception.   It identifies constraints to vaccine production and quality as they relate to CBPP vaccine and suggests focal points for discussions on improvements required for further enhancement of CBPP vaccine quality.

2   Nature of present CBPP vaccines

The CBPP vaccines currently used in Africa are freeze dried broth cultures of live attenuated *Mmm*SC representing either strain T_144 or its streptomycin-resistant derivative, T_1SR. Strain KH_3J and KH_3J-SR have been abandoned largely on account of their poor immunogenicity. Ten laboratories are actively involved in CBPP vaccine production while one occasionally manufactures vaccine depending on national demand.   Of these, nine laboratories submit all or some of their products to the PANVAC vaccine quality control

testing. The manufacturing process is based on the seed lot system and the master vaccine seed stocks are held at PANVAC and CIRAD–EMVT.

3 Vaccine quality control

The OIE norms for CBPP vaccine (1) require that the vaccines, which should have been produced under good manufacturing practice based on a seed lot system, should be tested for sterility, viable mycoplasma content, seed strain identity, innocuity, safety and efficacy. Due to cost considerations *in vivo* tests are not routinely carried out at PANVAC. The equipment, materials and methods used in performing vaccine quality control as currently carried out at PANVAC are as described in the standard operating procedures for the quality control of CBPP vaccine (5). Figures 1 and 5 show the number of batches tested and the quality control results of monovalent CBPP and combined CBPP–Rinderpest (CBPP component) vaccines tested at PANVAC over the past five years.

It is evident that the major causes of vaccine batch failure are inadequate potency (low titre) and sterility (contamination with bacteria or fungi). In the case of combined vaccine mycoplasma and/or acholeplasma contamination also constituted a significant cause for vaccine rejection (Figures 3 and 8). Figures 2 and 6 depict evolution of overall pass rates while the annual mycoplasma titre means are shown in Figures 4 and 7.

Figure 1. Monovalent CBPP vaccine: Overall result
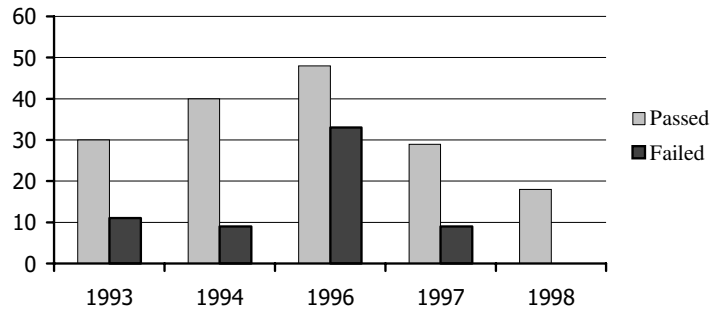
	1993	1994	1996	1997	1998
Passed	30	40	48	29	18
Failed	11	9	33	9	0

Figure 2. Monovalent CBPP vaccine: Pass rate %
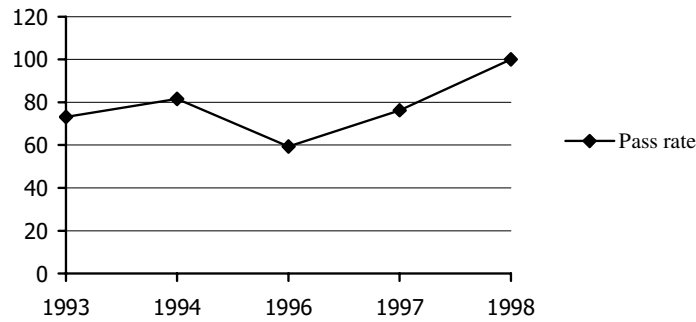
	1993	1994	1996	1997	1998
Pass rate	73·17	81·63	59·26	76·32	100

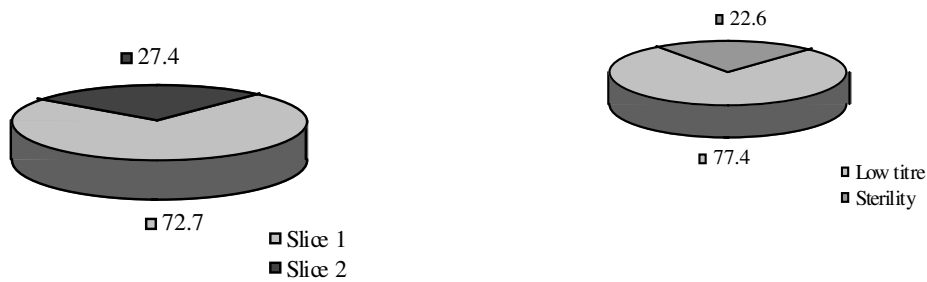**Figure 3. Monovalent CBPP vaccine: Reason for failure**

Figure 4. Monovalent CBPP vaccine: Mean titre/dose
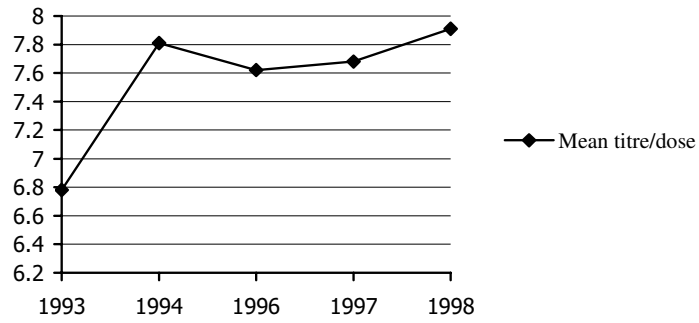
	1993	1994	1996	1997	1998
Mean titre	6·78	7·81	7·62	7·68	7·91

Figure 5. CBPP–rinderpest vaccine: Overall result
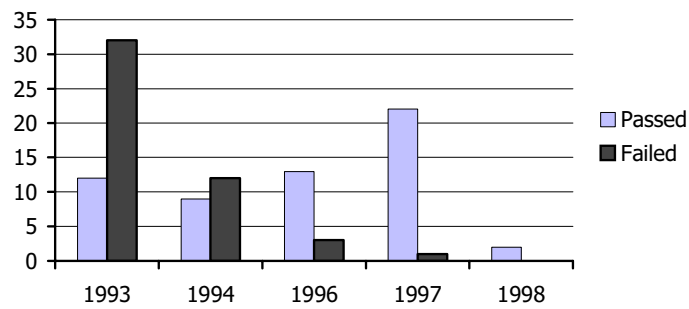
	1993	1994	1996	1997	1998
Passed	12	9	13	2	2
Failed	32	12	3	1	0

Figure 6. CBPP–rinderpest vaccine: Pass rate %
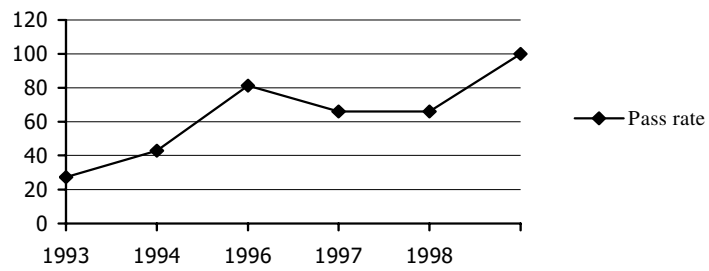
	1993	1994	1996	1997	1998
Pass rate %	27·27	42·85	81·25	66·66	100

Figure 7. CBPP–rinderpest vaccine: Reason for failure (%)

45%

55%

Passed   Failed

Low titre 55.6   Sterility l5.6
OtherMycos 8.9   L/totre+O/myco 20.0
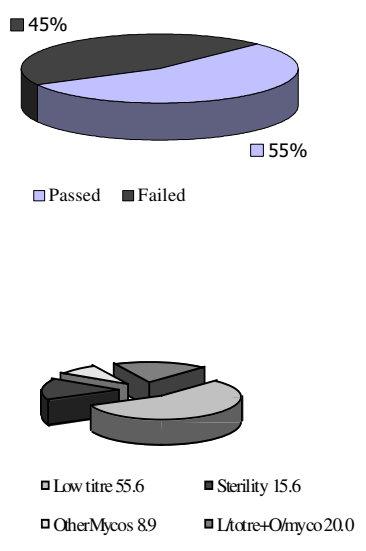
Figure 8. CBPP–rinderpest vaccine: Mean titre/dose
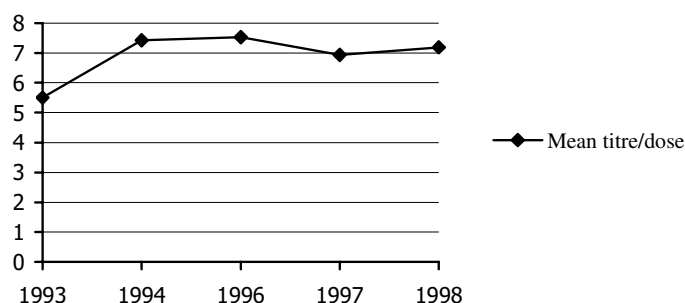
	1993	1994	1996	1997	1998
Mean titre	5·5	7·42	7·53	6·93	7·19

4   Global issues of CBPP vaccine quality

An examination of the quality control test procedures, results and observations pertaining to the performance and use of CBPP vaccine in the field raises certain key issues that ought to be addressed with the ultimate objective of improving the quality of this vaccine. Some of these matters include:

4.1   Vaccine seed strain histories

Perusal of producer vaccine seed strain data sheets shows that there is wide variation in the passage levels of manufacturers seed passage levels. For instance T_1SR vaccine strains used in laboratories D7, M8 and A8 are indicated to be of passage levels 45, 44 and 46 respectively. Such disparities in producer seed passage levels mean that final products from different laboratories will contain mycoplasma at varying passage levels and they may therefore confer unequal protection to immunized animals. The seed passage histories given are also rather puzzling because to obtain T_1SR, the $T_1 44$ (master seed strain at passage level 44) parent is said to be have been subjected to 3 successive subcultures in medium containing increasing concentration of streptomycin (4). Consequently, the theoretical T_1SR master seed lot should be at passage level 47. Recently a common $T_1 44$ seed stock was prepared at PANVAC and tested in vitro at CIRAD-EMVT and PANVAC. The *in vitro* tests were satisfactory and the seed stock has been supplied to all CBPP vaccine producer laboratories in Africa.

The above would seem to indicate the need for a clear statement to be made on internationally-agreed seed strain(s) for CBPP vaccine in Africa.

4.2   Vaccine batch failure

The chief causes of failure of CBPP vaccines are insufficient potency (low mycoplasma titre), sterility (bacterial and/or fungal contamination) and other mycoplasma contamination particularly with regard to combined CBPP–rinderpest vaccines. The vaccine performance figures presented show that whereas contamination (bacterial, fungal or mycoplasma) used to be a big problem at initial stages this has decreased considerably. This would suggest that vaccine producing laboratories are now paying closer attention to implementation of the principles good laboratory practice (GLP), particularly aseptic techniques in their manufacture operations.

However, obtaining sufficiently high mycoplasma titres in the final product still remains a major drawback. The OIE minimum titre requirement for CBPP vaccine of ten million viable mycoplasmas per dose is used by PANVAC as the passmark upon which a vaccine batch is accepted or considered to have failed the test for *in vitro* potency. It is further recommended that vaccine producers strive to release vaccine with titres of 10^8 mycoplasmas per dose on account of possible losses during post lyophilisation storage, transportation and usage. From 957 titrations representing 319 batches of monovalent and combined CBPP–rinderpest vaccine a mean titre of *7.24* mycoplasmas per dose was obtained. This is just marginally above the minimum required titre.

It is therefore necessary that ways of increasing mycoplasma yield in CBPP vaccine cultures be devised. One possibility would be the evaluation of use of fermenters. At the same time titre losses during downstream processing of vaccine (including optimised lyophilization cycles).

4.3   Vaccine seed strain identity tests

The tests currently used to determine and confirm the identity of CBPP vaccine seed strains include: colony characterization (size and morphology), sensitivity to digitonin, biochemical characterization, agar gel immunodiffusion, growth/metabolic inhibition as well as the test for streptomycin resistance. A critical examination of each of these tests reveals certain weaknesses particularly with respect to their specificity and sensitivity. For instance:

(i)   *Growth inhibition:* This is the OIE reference test prescribed for the confirmation of seed strain identity. However, in this test mycoplasma colonies breaking through the zone of inhibition are often encountered particularly with T_1SR and less so with T_144. Triple cloning of such breakthrough colonies and subsequent examination of the clones using the agar gel immunodiffusion suggests that they are *M. mycoides* subsp. *mycoides*. It is not clear whether these observations represent some kind of inherent antigenic shifts or other mechanisms in operation. But from vaccine quality control stand point this means that the test is not sufficiently sensitive.

(ii)   *Colony characterization:* The T_1SR and T_144 CBPP vaccine seed strains exhibit typical colony morphology characteristic of the genus mycoplasma i.e. fried-egg or mammillary appearance. The species *M. mycoides* subsp. *mycoides* has been classified as either small colony (SC) or large colony (LC). The SC diameter should be less than 1 mm whereas the LC diameter is usually more than 2 mm. At PANVAC observations on the colony sizes of vaccine strains show that both

the T_1SR and T_144 seed strains have mixed colony sizes ranging from 0.2 to 1.5 mm diameter on day 14 of incubation. But the average colony size is less than 1 mm in diameter. Cloning of either "small" or "larger" colonies of either vaccine strain and reseeding of the clones on agar gives rise to colonies of varying sizes with a mean of less than 1 mm in diameter. This therefore means that vaccine mycoplasma colony size is not a stable characteristic and that the term SC is a relative one referring to means of colonies of varying sizes. Consequently this test does not provide a useful mechanism of identifying CBPP vaccine seed strains.

(iii) **Streptomycin resistance**: The test for streptomycin resistance is recommended for use as a marker for T_1SR vaccine seed strain. However, investigations at PANVAC have shown that some T_144 are partially resistant to streptomycin at the OIE prescribed concentration of this antibiotic (1 mg/ml). It appears that streptomycin resistance is easily acquired by mycoplasmas and this may also suggest the possibility of the existence of streptomycin-resistant wild strains of *Mmm*SC especially following therapeutic administration of this antibiotic. Indeed some CBPP agents that are resistant to streptomycin have been isolated at CIRAD–EMVT (Thiaucourt, personal communication). This makes the test for streptomycin resistance rather imprecise in the identity of streptomycin resistant strains.

(iv) **Agar gel immunodiffusion and biochemical characterization**: These tests, as currently prescribed and applied, suffer from the disadvantages that they cannot discriminate amongst members of the *M. mycoides* "cluster" of mycoplasmas. Hence their usefulness in quality control tests for identifying CBPP vaccine strains from other closely related mycoplasmas is limited.

The examples given above demonstrate that the tests currently used to identify CBPP vaccine seed strains suffer from the inability of these tests to conclusively discriminate wild strains of MmmSC from those employed as vaccine strains. Consequently, this situation therefore calls for the development and standardization of tests that are able to distinguish vaccinal strains of mycoplasma from wild biotypes. In this regard recent advances in the study of some aspects of the molecular biology of MmmSC would seem to provide a sound basis upon which more accurate tests for identifying vaccine strains could be standardized (6, 7, 8, 9).

5 Safety

A safe vaccine is one that does not cause undue local or systemic reactions when used as recommended. Strictly speaking none of the currently used CBPP vaccine strains meets this requirement. As an illustration the following points regarding T_1SR and T_144 should be pointed out:

T_144: Still retains some measure of residual virulence particularly to certain cattle breeds. Subcutaneous inoculation of this strain causes unpredictably variable post vaccinal reactions which may lead to death in a small proportion of vaccinated animals (Table 1). It is, however, a good immunogen protecting vaccinated cattle for at least one year.

T_1SR: Compared to T_144, T_1SR causes fewer post vaccinal reactions. Original cattle efficacy tests on this strain in Senegal which involved inoculation of 100

million mycoplasmas per animal indicated that the strain is as efficacious as T_144 (10).

These observations strongly suggest the need to devise ways of making the current vaccine strains less virulent. It is known for example that T_144 causes fewer reactions when it is inoculated subcutaneously into an area of dense connective tissue like the tail tip (11, 12, 13). Another way of overcoming the safety problem would be to attenuate the organisms by subjecting them to further passages in broth cultures. However, the risk of subculturing mycoplasmas is that through some ill-understood mechanisms, they lose their immunogenic potency. It may therefore be more prudent to first of all investigate and define the virulence factors in the vaccine strains and on the basis of which attempts could be made to eliminate the virulence via molecular biology procedures such as deletion of the section of the mycoplasma genome that is responsible for pathogenicity.

6 Efficacy

Currently, the best procedures for testing the efficacy of CBPP vaccines is by virulent contact challenge of vaccinated animals. Such tests are not only expensive but require long periods of time. Besides, cattle challenge experiments are not always easily reproducible. The present CBPP vaccine strains confer protection for only about one year. But such protection is only solid in about 80 percent of vaccinated animals. It is because of this reality that immunisation against CBPP should be administered repeatedly at short intervals. Unlike many live vaccines, an effective immunization against the disease requires inoculation of a heavy dose of mycoplasmas (13).

Additionally, field observations in newly infected areas of Africa seem to suggest that vaccines based on T_1SR may not be as potent as would be expected of T_144 in prevention of CBPP. It is not quite clear whether these observations are due to the nature of the vaccine strain itself or the emergence of wild strains of *Mmm*SC which are antigenically divergent from the vaccine strain. It may also be that vaccines which did not contain the required number of mycoplasmas were administered; a situation which could easily arise from mishandling of vaccine during storage, transportation and/or use.

This situation therefore calls for the revalidation of the immunogenic potency and efficacy of the current vaccine strains. To facilitate this cattle experiments are in progress in Cameroon, Kenya and Namibia aimed at comparing the efficacy of T_144 and T_1SR vaccines. With the aim of cutting down on the cost of vaccine batch testing it is necessary to investigate cheaper methods of assessing the potency of CBPP vaccines e.g. in appropriate laboratory animal models (14) and/or in vitro techniques with acceptable correlation to in vivo results. In view of the short duration of immunity conferred by current CBPP vaccines there is need also for development of alternative vaccines which are safer and able to induce better protection to immunized animals. In order to do so, a better understanding of the mechanisms of immunity and pathogenesis in CBPP is essential.

Table 1. Observations on recent post vaccinal reactions using $T_1$44

Location	Cattle Breed	No. Vaccinated	No. Reacted	No. Dead	% Reactors	% Mortality
Northern Namibia	NG	500 000	6	0	0·001	0
Southern Ethiopia	Boran	452 069	517	18	0·11%	0·004
PANVAC	Crosses	33	6	0	18·8	0
KARI/Muguga*	Crosses & Boran	41	18	0	43·9	0
Rwanda	Crosses	30554	4027	NG	13·18	NG
Tanzania, Kagera region	Crosses	3050	31	31	1·02	1·02

NG – not provided.

* Out of 41 cattle vaccinated at the same time with T_1SR, 5 (12·2%) showed milder reactions.

7   Stability of CBPP vaccines

M. mycoides subsp. *mycoides* SC is easily inactivated by heat. CBPP vaccine handling therefore requires the maintenance of a cold chain right from the end of lyophilization to inoculation into target animals. This requirement not only raises the cost of vaccination programmes but it is also risky because refrigeration systems in Africa do not always function in a reliably consistent manner due to frequent power interruptions. Means of the thermostabilization of CBPP vaccine would greatly alleviate such problems.

Could this be achieved by an extended lyophilization cycle using suitable stabilizer as has been demonstrated for rinderpest vaccine? It would also be relevant to suggest that examination of any thermotolerant clones within the current vaccine strains could be investigated. If present, selection and use of such clones as heat resistant vaccines could be attempted, as has been shown for newcastle disease, heat tolerant vaccines. It is also conceivable that using recent advances in molecular biology thermotolerant recombinent vaccines could be developed using thermotolerant vectors such as the pox viruses.

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VACCINES AND VACCINATION: CBPP VACCINE REQUIREMENTS AND LOGISTICS

Peter Roeder

1 Introduction

A detailed consideration of logistical and vaccine requirements should obviously be influenced by the use to which they are to be put — equilibrium control, progressive control or eradication. This brief paper considers generic issues, hopefully to stimulate some discussion.

The progressive control of CBPP in Africa, with eventual eradication if this proves feasible as it has elsewhere in the world, requires a much improved penetration of the livestock herds to achieve a sustained elevation of herd immunity as well as requiring improved zoo-sanitary control. Both require a major effort to improve the veterinary–farmer interface and strengthen veterinary services in the medium to long term. It must be considered that global elimination of rinderpest, so near that it requires only a final thrust, would be a major advantage to create an enabling environment for CBPP control in Africa by freeing resources to undertake the required strengthening. If the rinderpest eradication goal is not reached and Africa reverts to endemicity following a new pandemic resources will again be drained by rinderpest control.

As with other contagious diseases, mass vaccination campaigns have an important role to play at the initial stages of a revitalised CBPP control programme but their use must be viewed as a temporary expedient before other more focussed strategies are employed, combining case finding with livestock movement control and focussed immunization. The herd immunity levels obtained through annual pulsed immunization, such as undertaken through the institutionalized annual vaccination campaign approach, could almost certainly be improved upon by repeated cohort immunization timed at the farm level rather than at national level — at least in more sedentary farming systems. It is necessary to design specific control strategies for different farming systems with reducing the weight of infection in extensive migratory farming systems an important element.

Essentially there are three types of CBPP problem areas which need to be addressed:

(1) The relatively remote, extensive, pastoral areas of Africa in which the disease is endemic and from where it repeatedly spreads in trade (including informal forms of trade such as rustling and cultural exchanges) which is insufficiently subject to zoo-sanitary procedures to constrain the spread of CBPP;
(2) The sedentary traditional cattle breeding, rearing and production systems — mixed farming and peri-urban farming systems;
(3) The relatively developed ranching systems which are at risk of infection in three ways:
- introduced fattening and transient trade stock, from the traditional extensive and sedentary pastoral systems, which are bought in with disease;
- breeding and other fattening stock contaminated from these;
- contamination from local communities.

Each of these have implications on the vaccination strategies, logistics of delivery and the vaccines required.

2 Logistics: improved access to communities and their cattle — application of vaccine to give high and sustained herd immunity

Conventional control programmes have relied recently more on vaccination to suppress epidemic events in newly-infected populations, which is of course desirable, than on reducing the weight of infection in endemic areas and the rate of spread from them. It is clear that the source of infection needs to be addressed more energetically. How can this be done?

(1) Establishing Community-based Animal Health Worker (CAHW) programmes with full community ownership — the methodologies are becoming well-defined;

(2) Developing private sector contractual arrangements — again, there are a growing number of precedents for this;

(3) Ensuring that the nomadic populations which span territorial boundaries and inter–country trade are taken fully into account and addressed adequately in control programmes — mechanisms have been developed both in Africa and Asia but need to be further developed. Methods of mapping and predicting movements are required.

To be effective, all of these activities require sustained extension programmes to enhance awareness of all sections of the veterinary, livestock owner — improving the veterinary/farmer interface — and trader communities.

In extensive pastoral communities heavy reliance will undoubtedly continue to be placed on pulsed vaccination programmes but these need to be supplemented by additional on-demand vaccination targetted especially at younger age groups. For more accessible communities on-demand vaccination combined with repeated national immunization periods could be contemplated. Whatever the approach it is clear that cost recovery or at least cost sharing will need to be a significant component of a strategy given the current status of public funding. However, cost recovery is not without its own problems and experience has suggested that cost recovery in CBPP control vaccination programmes can be detrimental by reducing vaccine uptake. It is perhaps more appropriate as a holding action in an equilibrium control/endemicity situation rather than for progressive control. In these circumstances CBPP control can be viewed as a component of routine health maintenance, at least during the initial stages of reversing the current trend of disease expansion. Later, mopping up procedures may be viewed as having a greater element of public good with a different attitude to apportioning costs.

In all farming systems there is a need to evaluate the likely effects of different types of intervention and mathematical modelling provides a valuable tool to do this. Even relatively simple deterministic models could provide very useful information with which to compare different strategies as well as provide cost–benefit comparisons.

3 Shortcomings of current vaccines

What are the problems faced with currently available vaccines? These are essentially:

3.1 Poor immunogenicity

- *Continued sub-clinical circulation of the agent in the face of immunization*

  This is perhaps inherent in the immunity induced by currently available vaccines, however low the level of continuing contamination of the livestock population, and requires further study — again modelling has a role to play here. It may be that vaccination alone can never interrupt the cycle of transmission making eradication an unachievable goal without additional actions.

- *Poor immunogenicity — short duration of immunity*

  Immunogenicity of vaccines is lost rapidly after reconstitution. Could this be improved by different reconstitution fluids? It could certainly be improved in the case of the smaller traditional producers and in more extensive pastoral communities serviced by CAHW programmes by providing vaccine in small dose vials.

  The use of short duration of immunity vaccines combined with annual pulsed vaccination inevitably leads to extreme fluctuations in herd immunity levels. The lapses in control resulting inevitably lead to a failure to interrupt the cycle of transmission of infection and continuing endemicity. Seromonitoring to guide the assessment of vaccination programmes and periodicity of immunization is a management tool that needs to be developed and applied. Obviously improved vaccines which confer complete protection for longer periods would greatly enhance CBPP control.

  Could improved vaccines result from enhancing the effective antigen mass content of conventional vaccines? Perhaps improvements could result if the antigenic elements responsible for protective immune responses could be identified, if growth and production of the relevant antigen could be maximised, if the antigen could be purified and if pre-lyophilization losses could be reduced.

3.2 Cold-chain dependence

Cold chain dependence is a constraining factor on the effective use of vaccines, especially in the more remote traditional pastoral areas. A thermostable formulation of conventional or improved vaccine could have considerable advantages just as it has had in the case of rinderpest. This could possibly be achieved through the use of improved stabilizers for lyophilization and improved freeze-drying cycles or through techniques such as trehalose dessication. Improvements in the cold chain itself are required and could result from increased use of cold-chain monitoring devices and development of improved vaccine storage containers for field use, for example using water cooling.

3.3 Adverse vaccine reactions
These appear to result from:
- breed–related susceptibility;
- poor vaccination technique — overcoming this requires improved training and motivation of vaccinators;

- adventitious bacterial contamination of vaccines either at source of production or during use — to be avoided by:
  - insistence on use of quality-assured vaccines;
  - training of vaccinators in aseptic technique;
- immune reactions occurring when infected cattle are vaccinated – farming communities need to be fully aware of this and consideration needs to be given to how these reactions can be minimised or treated to reduce losses.

3.4   Cost

Eventhough conventional vaccines are relatively inexpensive to produce the costs of vaccination are high to be effective because of the need for repeated vaccination to achieve a sustained protective immunity. Novel vaccines are likely to be more expensive, at least initially, and must have greatly enhanced efficacy if their use is to be attractive, cost-effective and competitive.

4   Vaccines required

What do we need?
- low cost
- easy to produce
- small dose vials as well as large dose
- thermostability
- innocuity — all breeds
- high immunogenicity
- long duration of immunity exceeding one year
- ability to break the cycle of transmission i.e. complete protection from infection, not just disease, and shedding.

What are the prospects? This meeting will review the state of progress in development of novel vaccines. It will be clear that there are still many avenues to pursue, hopefully to be based on a sound understanding of pathogenesis, immunity and antigenicity of the causal organism.

VACCINES AND VACCINATION: THE IMPACT OF WITHDRAWING RINDERPEST VACCINATION ON CBPP CONTROL

Walter Masiga, Paul Rossiter and Rene Bessin

Summary

The mass vaccination campaigns carried out by PARC against rinderpest also vaccinated cattle against CBPP. However, many African countries have recently stopped vaccination against rinderpest as the preliminary step towards a final declaration of freedom from rinderpest infection. There is now a reported increase in the incidence of CBPP in several countries and it has been suggested that this is due to stopping the campaigns against rinderpest. Most of the countries reporting increased incidence of CBPP either stopped rinderpest campaigns too recently to be able to measure their effect yet, or are not reporting disease outbreak data to support their claims. The best evidence for the relationship comes from Ethiopia where CBPP is now a problem in the highlands in which vaccination campaigns against rinderpest ceased some four to five years ago. There are several possible reasons why effective CBPP vaccination coverage may not have been maintained after stopping mass campaigns against rinderpest.

1   Introduction

Vaccination against contagious bovine pleuropneumonia (CBPP) has frequently been carried out in conjunction with vaccination against rinderpest. In many countries in West Africa, in Ethiopia and occasionally in other countries this was done using a combined vaccine such as Bissec. In East Africa cattle are more often inoculated separately with monovalent rinderpest and CBPP vaccines. These practices were widely used during the Pan African Rinderpest Campaign (PARC) which began in 1986. The mass campaigns were successful in many areas. Rinderpest has not been reported from West Africa for 10 years, from Central Africa for 12 years, and from Ethiopia for nearly three years. As a result PARC and other partners have encouraged Member States in these areas to join the OIE "Pathway" which is designed to help prove the absence of rinderpest virus from a nation's cattle. The essential first step on the "Pathway" is to stop rinderpest vaccination in a country or zone that wishes to make the initial declaration of "Provisional Freedom from rinderpest disease". In the last two to three years therefore, several countries in West Africa, and also Ethiopia and, very recently, Tanzania in East Africa, have ceased vaccination against rinderpest. Concomitant with this have been reports of an apparent increase in the incidence of CBPP in several of these countries, with the implication that this is due to the decline in rinderpest vaccination campaigns which also delivered CBPP vaccine. Obviously, it is not the absence of rinderpest vaccine that is causing the reported resurgence of CBPP but a possible decrease in the number of CBPP vaccinations. This paper reviews some reported figures to see whether there has been a decrease in CBPP vaccinations, and if so, whether this is related to the cessation of joint rinderpest/CBPP campaigns or other factors.

2   Evidence for decreased vaccination against CBPP

An accompanying paper presented earlier in this meeting indicated that without more data it is probably still too early to be sure of the extent to which there is a real increase in the incidence of CBPP in the endemically infected countries that previously had kept the disease under good control by vaccination.

The number of reported outbreaks of CBPP and associated mortality in some West African States and in Ethiopia are shown in Table 1. The numbers of animals vaccinated against CBPP in these countries is shown in Table 2. These countries have been selected because they have either completely stopped or greatly reduced vaccination against rinderpest, and because they usually used bivalent vaccine.

It is difficult to draw any firm conclusion from these preliminary national statistics, especially as several countries only completely ceased vaccination in 1997, which should lead to their cattle populations becoming susceptible in 1998 and 1999 unless immunization is maintained. Fortunately, most countries are continuing vaccination with monovalent vaccine or are proposing to do so. Nevertheless, it is possible to make some preliminary observations from the data presented. The data in Table 1 suggests that over the past ten years there appears to have been a reported increase in the incidence of CBPP in Ethiopia and Burkina Faso.

Table 1. The number of outbreaks of CBPP and recorded mortality selected PARC Member States (1987-1997)

Country	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Burkina Faso	10*	21	7	9	4	6	8	7	29_{115}	28_{221}	29_{93}
Côte d'Ivoire	7_{41}	9_{77}	14_{80}	18_{197}	16_{120}	9_{79}	12_{68}	7_{19}	12_{48}	11_{27}	10
Ethiopia	96	1	+	3	6	1	+	10	14	55_{295}	18_{93}
Guinea	?	105	49	38	82	49	20	17	48_{79}	30_{9}	
Mali	4	5	15	37	12	15	20	21	11_{294}	12_{47}	6_{83}
Niger			0	5	8	13	4	8	3_{13}	8_{23}	9_{3}

$N_X - x$ = reported mortality (number of animals that have died); shaded cells indicate the year in which rinderpest vaccination was completely stopped.

* The data is taken from official reports contained in the OIE, in the FAO/OIE/WHO Animal Health Yearbooks, and in PARC reports.

Table 2. The number of vaccinations (in millions) against CBPP in selected PARC Member States   (1987-1997)

Country	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Burkina Faso									1·42 *	1·58	
Côte d'Ivoire		0·06	0·89	0·98	0·78	0·93	0·98		0·73	0·95	
Ethiopia		11·1		21·1	8·15	6·01	4·06	4·43	4·33	4·95	2·82
Guinea					0·56	0·10	0·39	0·10	0·47		
Mali							1·65	1·23	1·52	1·18	1·90
Niger	1·55	1·05	1·09	1·34	0·93	0·62	0·50	0·63	0·34	0·63	0·84

* Data from PARC documents and reports

In Ethiopia the upsurge in incidence coincides with the time at which mass rinderpest and CBPP vaccination was stopped in the highlands and attention has been drawn to this in several PARC Ethiopia reports. The new outbreaks are mainly in the highlands and are caused by livestock movement from the endemically infected lowlands. In the other countries there is no clear indication of overall increases in the reported incidence of the disease even though these countries now view this disease as the most serious transboundary disease affecting their cattle populations. This was clearly restated at the recent 12th PARC West and Central Africa Coordination Meeting in Accra at which several participating Member States reported that CBPP was now a serious problem. OAU/IBAR will continue epidemiological analysis of this relationship over the next few years in order to determine the true correlation between vaccination and the incidence of CBPP. Recognizing also that such "macro-epidemiological" analysis cannot account for focussed tactical vaccination of high-risk populations, OAU/IBAR will also analyze the vaccination versus disease incidence relationship in various national sub-populations of cattle. In addition, since the figures on mortality presented in Table 1 do not constitute significant economic loss for the continent, these studies will be made in sufficient detail to provide the data needed for a thorough investigation of the true economic costs of CBPP in Africa.

There is still insufficient data to categorically confirm that there is an increased incidence of CBPP throughout equatorial Africa resulting from the cessation of donor supported mass rinderpest vaccination campaigns. Nevertheless, the evidence from some individual countries indicates that this may be the case.

3 Possible causes of a decline in vaccination against CBPP

3.1 Reduced delivery of government animal health services

National mass vaccination campaigns against any disease are expensive and, unfortunately, often beyond the budget of most African countries. Without external support many countries are now unable to mount mass vaccination campaigns. In the past, the PARC campaign ensured that mass campaigns were financed to combat and eradicate rinderpest. Now that PARC has moved into the eradication phase which does not fund vaccination campaigns, many countries are unable to mount effective campaigns against CBPP using their own resources, despite having established revolving livestock health funds under PARC.

3.2 Cost recovery for vaccination

Another factor to be considered is cost recovery. Many countries now ask the livestock owner to contribute to the cost of the vaccination of his stock. In some cases this is the cost of the vaccine, which is not high, but in others it includes a variable proportion of the delivery costs, which are significant. Any detailed analysis of the possible reasons for reduced immunity to CBPP in Africa must consider this issue.

3.3 The role of $T_1 44SR$

A vaccine related issue that may also have contributed to a reduction in CBPP herd immunity levels is the use of CBPP vaccine using the $T_1 44SR$ substrain. During 1995 and 1996 there were a number of reports suggesting that some vaccination campaigns using this vaccine had not immunized cattle sufficiently and that they were not protected against the disease. This

was a new phenomenon since it is well established that T_144SR vaccine usually gives satisfactory immunity. Various suggestions were made to explain this apparent failure. One, that some batches of T_144SR may have lost their immunogenicity. Two, that T_144SR was not effective in the face of the epidemics of CBPP that were emerging in Africa at that time. Three, that following freeze drying, the vaccine might sometimes contain insufficient titres of living organisms to produce solid immunity that would last until the next campaign.

In the meantime, there were international recommendations that countries should use CBPP vaccine prepared from the parent strain T_144 rather than T_144SR. This in turn brought its own problems. Some countries may have had to delay their campaigns whilst obtaining stocks of T_144, and others would have been left with stocks of T_144SR that they needed to utilize before purchasing T_144 vaccine. More importantly, T_144 vaccine was reported, in some circumstances, to cause post–vaccinal reactions at the site of inoculation if administered incorrectly. Although this is probably due to incorrect administration as opposed to the vaccine itself, it has nevertheless lead to reluctance on the part of some veterinary departments to use this vaccine widely. More significantly it has also lead to reduced acceptance by the livestock owner.

PARC is currently funding an experiment to investigate the immunogenicity of T_144SR in order to try to resolve this very important issue. Since 1995 PARC has also funded a joint research project between several European and African laboratories that is developing and testing a completely new type of vaccine against CBPP. The final experiments in cattle should be concluded in the near future. These initiatives, and others, will hopefully lead to new effective vaccines against CBPP that give much longer lasting immunity than is presently obtained without the risk of using live and sometimes potentially pathogenic, organisms.

4   Conclusion

With the exception perhaps of Ethiopia, it is too early to be sure that the increase in CBPP reported from several of the endemically affected countries in Africa is the result of stopping vaccination against rinderpest. OAU/IBAR will continue to monitor the levels of vaccination against CBPP and correlate these with vaccination coverage before stopping rinderpest campaigns, and with more accurate data on disease incidence and distribution.

RESEARCH NEEDS: MYCOPLASMOLOGY

J. B. Bashiruddin

1   Introduction

The past ten years has seen the re-emergence of CBPP in areas previously free of the disease for several decades. In several Southern parts of Europe it has been or is being eradicated from areas in which it was reintroduced probably from remanant pockets of disease within Europe. This has provided the impetus and the opportunity to examine the disease with current scientific tools. Important funding, in part, has come from the European Union with research contracts such as 51003594917 (DGVIII) and FAIR1–CT95–0711 (DGVI), and coordination programmes such as COST 826, and these have been backed by funding from the individual countries involved. These cooperative efforts have increased dialogue between experts and fueled better collaboration between institutes. Much of the research has been into the molecular biology and biochemistry of *Mycoplasma mycoides* subspecies *mycoides* SC (*Mmm*SC), but only some has been directed towards the host. Nevertheless, it has led to the development of several serological, and molecular detection and identification systems, and these tests have made some progress towards rapid or easier diagnoses of CBPP.

2   A selection of questions to be answered

Many questions about the basic biology and the mechanism of disease production by *Mmm*SC remain unanswered. For example:

- Mucus membrane pathogens like *Mmm*SC must exist in close association to epithelial cells, so how do *Mmm*SC cells attach to host cells? The mechanisms of adhesion are known for some human mycoplasmas, e.g. *M. pneumoniae*, and for several animal mycoplasmas, e.g. *M. hyopneuminiae* and *M. pulmonis*, and the discovery and elucidation similar mechnisms, if they exist, should be pursued.

- How do they bring about disease? Is it toxin mediated? Is it a host reaction? Is it both of these? Some information exists about the capsular galactan of *Mmm*SC; its chemical structure is known to be similar to normal pneumogalactan found in the bovine lung, but its exact role in virulence has not been clarified. Differences in the amount of galactan found *in vitro* are thought to infect virulence, but, in culture surface carbohydrate (CHO) production is a positive function of the rate of growth e.g. an increased growth rate results in the production of more galactan, and therefore these CHO differences in strains may reflect differences in growth rates, only. Pure galactan is not immunogenic in itself, but it is said to become highly immunogenic when mixed with surface proteins. Thus, it is difficult to know the *in vivo* role of this component of *Mmm*SC. The recently-described ability of African *Mmm*SC strains to produce peroxide from glycerol *in vitro* may be significant. A haemolytic activity may simply be associated with peroxide production which is the major haemolysin of mycoplasmas, and some workers have sequencing of part of the putative gene responsible.
- What is the importance of immune modulation in CBPP? Challenge with whole mycoplasmas after administration of the immunologically inert galactan caused an

anaphylactic-like reaction in bovines indicating that some cell-mediated immune effects are most likely. Some experiments suggest that *Mmm*SC has an immunosupressive effect on the host. The occurrence or extracellular components, enzymes and superantigens, which induce immune reactions over humoral responses have not been explored to a great extent.

- What is the role of switchable immunogenic proteins? In several ruminant mycoplasmas e.g. *M. bovis* and *M. agalactiae,* antigenic variability is controlled by a family of genes which confer the organisms the ability to vary their surface protein and lipoprotein structures. Immune evasion is one of the postulated function of these and the exisitence of similar mechanisms in *Mmm*SC needs to be investigated.

- Why is pathogenesis supressed in some hosts and not others? There are reports of *Mmm*SC detections from small ruminants; isolations have been made in Africa, and serological and molecular detections have been reported from Asia and Europe. Besides the need to survey this situation to reconsider the *Mmm*SC host range, there is a need to understand the basic host interactions and pathogenic mechanisms of *Mmm*SC which enable it to be pathogenic in some situations.

- By what process does a chronically diseased animal become a healthy carrier? Some information regarding the course of infection and disease through the observation of natural infections and, more recently, experimentally infected animals is available. Time courses of antibody production and respiratory shedding of *Mmm*SC have been studied with African, European and Australian strains, but the early events of colonization and establishment of *Mmm*SC in the respiratory tract are not known, nor are the very late events which lead to the conversion of only some afflicted animals to healthy carriers. The even later mechanisms of reactivation and/or return to shedding of infectious material also requires study. Only more animal experiments may be able to increase knowledge in this respect.

The following focus on some of the current research, and in the case of proteome studies, future research, may point out some of the benefits which may come from the continued study of *Mmm*SC.

2   Genetic studies

Some of the tools of recombinant genetic technology, after a period of adaptation, are being applied to *Mmm*SC. New techniques such as PCR and newer *E. coli* host strains which supress the UGA stop codon have accelerated this area of work. They have enabled the task of sequencing the entire genome of *Mmm*SC to begin. Automation of many of the tasks means that a reasonable amount of sequence data has already been recorded. The aim is to sequence enough randomly-generated fragments to cover the genome 4 times and thus ensure accurate data over the entire genome. Up to now fragments eqvilalent to twice the length of the genome have been sequenced although not all of these sequences have been analyzed. In the small portion of sequences analyzed, the IS*1296* and sequences related to the translation machinery e.g. tRNAs, are more abundant than expected.

3   Genes to proteins

As data become available computational analyses will assign genes which resemble similar sequences in *E. coli*, or *Bacillus* or yeast to the sequences of *Mmm*SC. This has been done with *M. genitalium* and *M. pneumoniae*. It should be pointed out that the gene assignments are putative. Not always do assigned genes correspond to the said activities, e.g. an open reading frame (ORF) which resembles a gene for spore germination has been annotated for *M. genitalium*. The functions of all the genes in *E. coli* are not known e.g. genes *glpE* and *glpF* in the glycerol phosphate regulon have no corresponding enzymatic activity. So similar genes in mycoplasmas would also have unknown functions as well. About 10% of the *M. pneumoniae* gene has not been annotated. By comparison of the annotated coding capacities of the 2 mycoplasmas for which the entire genome has been sequenced, and estimate of 1066 ORFs may be estimated for *Mmm*SC. Not all are proteins or enzymes but it represents twice as much potential as *M. genitalium*.

4   Adding functions to proteins

The question is: What could all these proteins do? By polyacrylamide gel electrophoresis about 60 polypeptides may be seen. More than half this number are included in ISCOM particles. By Western blotting with sera from naturally-infected cattle about 20 bands can be seen. Again the question is: What about the rest of the proteins? One way of finding 'the rest' is also underway. Random fragments have been inserted into an expression system which results in the production of foreign peptides on the surface of a filamentous phage. These phages are marked so that only those which express foreign proteins may be recovered. Genes corresponding to expressed proteins may be harvested and analyzed. Specific sera may also be used to recover particles containing specific sequences to immunoreactive proteins for further analyses, and similar studies are also underway.

Another way is to look at all the proteins at once. That is to look at the full protein complement of the cell, or its 'proteome', separated by two dimentional gel electrophoresis. Protein species of interest may then be collected from gels and analyzed by micro methods and variations of mass spectrometry and other techniques of protein chemistry. The peptide fingerprints thus determined may also be matched to data bases to reveal similarities and to deduce their likely functions. Estimations of the minimum gene complement of *M. genitalium* already show errors. Peptide analysis showed that MDH and LDH activities, which are similar tasks, were performed by one enzyme by the virtue of one altered amino acid residue at the active site. These studies may be able to identify proteins before the genes have been identified. In many cases experimental evidence of activity will need to be shown before enzymes are recognized. There are gains to be made from the correlation of genes to observed proteins.

5   Concluding remarks

From these studies alone benefits will be gained in the acquisition of basic knowledge on *Mmm*SC. Genetic studies may further elucidate phylogeny, variability, and evolutionary trends. Genes for adhesins and toxins, enzymes and virulence factors may be identified. Protein analyses may identify differences between virulent and not so virulent strains, identify immunoreactive molecules, or molecules with immune modulating functions. This may aid vaccine design and development. As a result, pathogenic mechnisms may be better studied as well. Better diagnostic tools may be designed, and better prophylaxis may result

from tailored chemotherapeutic agents. Certainly, provided with adequate consistent funding, some interesting times lie ahead in *Mmm*SC research.

RESEARCH NEEDS: IMMUNITY AND VACCINATION IN RELATION TO CBPP

Bror Morein

1 Introduction

Vaccines have been a great asset to combat acute infections for more than 200 years. However, vaccines are virtually lacking against chronic and persistent infections. *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC) causes a chronic infection not to say persistent. The necessity of a microorganism to prevail in a host is to survive long enough to find a new host. As a consequence of this, it is not likely that a whole live or killed microorganisms would induce protective immunity against a pathogen causing chronic infection. The strategy is then to find the protective antigens and get rid of the ballast of antigens. Another strategy is to define and omit in the vaccine the molecules with capacity to modulate the immune response the wrong way allowing the pathogen to persist long enough to find another host. We have defined such an antigen in *Trypanosoma cruzi*. By removal of that antigen, named Ag123, protective immunity was induced in a laboratory animal model. The advantage of identification of protective antigens is that these are likely to be produced more economically cloned in a high producing expression system. It is also possible to look into groups of antigens and find out if any of those is a pathogenic factor. With regard to *Mmm*SC there is a large carbohydrate fraction named galactan probably in the form of glycolipids or glycoprotein. The latter would be against the dogma saying that bacteria do not have glycoproteins at least not in large amounts.

In Namibia I saw the very strong reactions after experimental infection of cattle with a virulent recent Namibian *Mmm*SC isolate. An interesting feature was the fast development of sequesters which also has been confirmed in studies from Cameroon. Such reactions should be characterized with regard to chemokines, inflammatory or preinflammatory cytokines. Lymphocyte products e.g. Th1 and Th2 type of cytokines should also be looked for. The fractions of the *Mmm*SC cell, i.e. in the first instance the protein and carbohydrate fractions, should be used with the attempt to provoke such reactions in cattle and laboratory animals. Subsequently, vaccine candidates should be constructed to study the feasibility to induce protective immunity with any of the fractions respectively by omitting any of the fractions.

2 Immunostimulating complex (iscom) is a carrier system for subunits

The iscom is a 40 nm particle in which the antigen(s) are incorporated together with an adjuvant component (immunomodulator). The iscom induce a strong Th1 type (IFN–gamma, and IL–2) of response but also Th2 type (IL–4 and IL–5). The iscom is a flexible carrier in which virtually any component can be exchanged, i.e. the antigen but also the adjuvant component. By exchanging the latter a different type of immune response can be induced. With other words immune responses can be tailor-made.

ISCOMs have been made with *Mmm*SC antigens. Very strong and longlasting antibody responses were induced both in mice and in cattle in Munich. In mice IgG1, IgG2a and 2b subclass responses were induced. In cattle, high IgG1 and IgG2 serum antibody responses and strong T–cell response measured by the lymphocyte proliferation assay. The

doses were low both with regard to antigen and adjuvant. In Namibia, animals were immunized with the same doses, but the antibody responses were low, only 3 animals showed similar magnitude of response as in Munich, 4 responded with low titres and 5 animals did not induce detectable response after the two immunizations. There might be high and low responders, but by increasing the dose of adjuvant the low responders might approach the responsiveness of the high ones. We have seen this in mice using Epstein–Barr virus gp360 as antigen. Despite the fact that the iscoms vaccine used did not confer detectable or only low antibody titres, it was felt that the concept of iscom warrants further examination as a possible candidate for a CBPP vaccine.

(1) Though the immune response in the 15 Namibian cattle vaccinated with the iscom preparation was rather poor, an indication for a protection was seen. In difference to the control animals where eight animals died during the course of the experiment, only one vaccinated animal died during the same time period. If one analyses the temperature reaction of the two groups, it further becomes apparent that the control animals developed considerably higher temperatures after exposure to CBPP infected animals hence to infection.

(2) While the experiment vaccine for both Munich and Namibia was based solely on membrane constituents of *Mycoplasma mycoides*, it was felt that cytoplasmic components should be tested on their antigenic role to stimulate a specific immune response.

The second experiment with ISCOMs is now on the way in Namibia using high doses of the adjuvant component in the iscoms. The first serological data indicate that the iscom immunized cattle have high antibody titres. It should be borne in mind that the level of immune response does not guarantee immune protection to the subsequent challenge infection. Therefore, the immune response should be immunologically characterized to sort out differences between protected and not protected animals.

RESEARCH NEEDS: DIAGNOSIS AND SURVEILLANCE

M. H. Jeggo

1 Background

Contagious bovine pleuropneumonia (CBPP) is a severe disease of cattle that has occurred throughout the old world. Whilst the disease has been eliminated from most of Europe and Australia through primarily the slaughter of affected animals, it still occurs in one country in Europe, Portugal, and is found in several Asian and many African countries. During the 1960s and 1970s the disease appeared to be on the decline but recent new outbreaks in Europe (Spain and Italy) and more recently in a number of African countries (Botswana, Tanzania, Zambia) have served to highlight again the importance of this disease, the losses it can cause and aspects of the disease that are still poorly understood.

The disease has been brought under control and subsequently eliminated in a number of countries through a process of identification and slaughter of infected animals, although this does not always work e.g. Portugal or is impractical e.g. Angola. In many endemic areas in Africa, control through vaccination has been the preferred option. This approach though rarely leads to eradication and is beset with technical difficulties. The vaccines available have varying degrees of side effects and the duration and extent of the immunity is highly variable and dependant on a number of factors, some known some unknown. Significantly, little is known of the process of protective immunity in CBPP, there is not a good correlation between the presence of specific antibody and protection and no meaningful test has been developed to evaluate cell-mediated responses and immunity.

Laboratory tests of known sensitivity and specificity are central to animal disease diagnosis and surveillance, to the development of effective vaccines and to the monitoring of the use of vaccines. The ability to detect the presence of the causal agent of CBPP, either through identification of the agent (or part of it) or the response of an animal to the agent (whether through vaccination or natural, infection) is a key factor in control and eradication of this disease. Whether the approach be movement control, slaughter of infected animals, vaccination or drug therapy without this ability none of these approaches can really work in the developing country situation. This paper sets out to describe current diagnostic assays and their limitations, and what research is needed to provide more appropriate assays for epidemiological studies (surveillance) and for the development and operation of effective control and eradication strategies.

2 Diagnosis of CBPP

A clinical diagnosis is made in the field and confirmed by a "laboratory" test. For the most part such tests are conducted in a laboratory although a number of "penside" tests that can be used in the field are available for particular diseases e.g. *clearview* for rinderpest. *No such assay is available for the diagnosis of CBPP in the field and should be a research priority.* Laboratory-based assays are equally critical for conducting disease surveillance, for developing vaccines and evaluating vaccination responses, for international trade in livestock and for assisting declaration of freedom from disease and the processes leading to this. Fundamental to diagnostic assays is validation for the purpose for which they are being used

and information on their sensitivity and specificity. Such criteria are not applicable to a clinical diagnosis and the assurance provided by the use of "validated" assays for confirmation of a diagnosis is the main reason for submitting a sample to a laboratory following a clinical diagnosis. *Very few, if any, of the assays currently being used to diagnose CBPP have been properly validated following the OIE Guidelines on assay validation and information on their predictive value (sensitivity/specificity) is very limited. Much research is needed in this area.*

2.2   Clinical diagnosis of CBPP

This should involve an examination of the sick animal, a post mortem and an evaluation of the outbreak. Both the presenting clinical signs and the post-mortem findings in cases of CBPP have been extensively described and have not varied significantly over the years. *No obvious research is required in this area although a full understanding of the ameliorating effects of chemotherapy and/or vaccination on the clinical symptoms and post mortem findings is worthy of further study.*

The characteristics of an outbreak of CBPP, the predisposing factors and the eventual outcomes of a CBPP infection within a population of cattle are far from clear. The occurrence of the "carrier" has been known for many years but the factors that lead to the development of this state and its significance in maintaining CBPP within a population is poorly understood. *More research on the epidemiology of the disease is clearly needed but central to this is the need for more effective and better defined laboratory tests.*

2.3   Laboratory confirmation of CBPP

2.3.1   Isolation of the causative organism

The identification of the aetiological agent of CBPP as *M. mycoides* subsp. *mycoides* small colony (SC) (*Mmm*SC) has been known for some time and its and nutritional requirements, isolation procedures as well as the physical and biochemical characteristics for the synthesis, culture and characterization have been fully described. While there are few problems associated with the growth of the laboratory strains of *Mmm*SC, in practice detection rates of strains by culture frequently underestimates the level of infection. This can be due to the relatively poor survival of mycoplasmas during transport, the poor adaptation of some fresh isolates to *in vitro* culture, and to the widespread use of antibiotics which can severely reduce the number of viable organisms in samples. Although much progress has been made recently in adopting a defined media, the ability of different laboratories to isolate the organism is still variable and a failure to isolate the causative agent must be viewed cautiously.

Final identification of mycoplasmas is usually made by growth inhibition (GI) or immunoflourescence (IF) tests which are carried out on agar. The tests are relatively specific and can distinguish the two species of *M. mycoides* but not small and large colony types. A rapid dot immunobinding test can be carried out with unpurified mycoplasmas extracted from broth cultures by a membrane filter in microtitre plates. The mycoplasmas are typed by adding specific sera and then visualizing the immunocomplexes by the addition of enzyme conjugate and appropriate substrate. Overall, this approach can give similar degrees of specificity as the GI and IF although it can give cross reactivity between bovine group 7 and *Mmm*SC. Its advantage is speed and suitability for testing large number of samples.

2.3.2   Antigen detection

Immunocytochemical test to detect *Mmm*SC can be used on tissue sections. Stained antigen is seen in the smaller bronchioles and alveoli and within the interlobular septa. Foci of stained antigen both in these areas and in lymphatic vessels, in thrombi within them and in the intermediate zones of the perivascular organizing centres are considered diagnostic for CBPP. Immunocytochemistry is labour intensive but very useful in animals that die suddenly from an acute infection.

Immunofluorescent staining can provide a less ambiguous diagnosis and using impression smears can be more sensitive and quicker than culture; the method can be improved using Eriochrome black as a counter stain.

2.3.3   Polymerase chain reaction (PCR)

The development of the PCR has provided a powerful tool which not only distinguishes the *M. mycoides* subspecies from other members of the cluster, but can also detect the DNA from small numbers of organisms present in nasal mucus, pleural fluid and pulmonary tissue.   This approach therefore offers great sensitivity with accompanying specificity.   However, care must be taken in the selection of the correct primer (s) and through sample processing great care must be exercised to avoid contamination and false positives. The use of nested primers will probably prove essential in avoiding some of the above and in giving the required specificity. *There is no doubt that there is great potential for role of PCR in diagnosis and molecular epidemiological studies but research is needed both in standardizing the technique itself, on building an array of primers that are fully characterized and on what a "positive" PCR result can mean in terms of the animal itself. One of the major impediments to successful epidemiological tracing of CBPP is the homogeneity of strains of MmmSC. Whilst the molecular epidemiology of CBPP is in its infancy, PCR is essential and the routine use of this in a standardized and quality assured manner may prove critical to control and eradication programmes in Africa.*

2.3.4   Serology

Numerous serological tests have been described and these include slide agglutination, complement fixation (CFT), agar gel precipitation, passive agglutination (PHA) and ELISA, both indirect and competitive. The OIE Prescribed Test remains the CFT, an assay which has stood the test of time, is fully standardized and has proved central to successful eradication programmes to date e.g. Australia.

The indirect ELISA has been shown to be too insensitive but the recent development of a monoclonal antibody based competitive ELISA is proving highly specific and detects only antibody to the field strains and not CBPP vaccine strains. Currently this assay is being validated in some ten African countries and compared with the CFT in animals of known history (CBPP free, naturally infected and vaccinated animals). *It is critical this research is completed as in many ways the ELISA is the preferred assay for serology. Many laboratories in Africa currently use this technique for rinderpest serology, the assay is relatively cheap, can be used to process many samples and lends itself to quality assurance. Whilst other assays might be considered worthy of more research efforts, the enormous value that has been seen in the rinderpest eradication programme through having a universally standardized ELISA used by all countries might well apply to a CBPP control eradication effort. This then would highlight the need to focus further research efforts in serology on*

internationally validating and standardizing an ELISA with the required level of sensitivity and specificity. The use of a defined antigen and a well characterized and freely available monoclonal antibody would be the two key elements for success in this area.

2.3.5 Cellular immunity

The consensus at present is that immunity is primarily cell–mediated. Although an assay to determine this is not required to confirm a clinical diagnosis, such an assay is vital for epidemiological studies, for further studies on the pathology of the disease, and for assessing the effectiveness of vaccines. An allergic skin assay has proved useful but can provoke an unacceptable skin reaction in immune animals and the sensitivity and specificity of the assay have not been characterized. *Initial work on a macrophage procoagulation assay (MPCA) proved promising but much urgent research is needed to develop an assay that correlates with immunity.*

3   Surveillance

Although the word surveillance can be variously interpreted, in this context it is loosely used to mean the conducting of epidemiological studies or the study of CBPP in populations of animals to:

- establish the prevalence of the CBPP;
- evaluate vaccination programmes against CBPP;
- evaluate or monitor control or eradication strategies for CBPP;
- to prove freedom from CBPP in a country or region during passage down the OIE CBPP Pathway.

To undertake the exercises above surveys are usually conducted nationally but can be linked to purposive and slaughterhouse sampling. In the case of CBPP slaughterhouse surveys and the identification of infected animals has proved particularly useful and have formed the cornerstone of a number of control programmes. Critical to any survey is an initial definition of what the survey intends to achieve and how this will be undertaken. *Most surveys of infectious diseases require laboratory assays with known predictive values and for CBPP research is critically needed in this area. One essential laboratory assay missing is the one that correlates with immunity. It is clear that cell-mediated immunity is the critical element in protection but no assay is currently available to determine this. Epidemiological research (based on such assays) is fundamental to the development of sound control and eradication programmes and a number of critical questions remain to be answered e.g.*

1. *What are the predisposing factors for the spread of CBPP?*
2. *What characterizes the CBPP carrier state and what risk does a carrier pose?*
3. *What is the duration of immunity from currently used vaccines?*
4. *How long does antibody last in either a vaccinated or naturally infected animal?*
5. *What is the duration of maternal antibodies?*

RESEARCH NEEDS: ROLE OF CHEMOTHERAPY IN CBPP

Abdelali Benkirane

Summary

In face of the recent upsurge of CBPP and the failure of available vaccines to confer a solid and long lasting immunity, chemotherapy has become a fact of life in attempting to curb the devasting effects of the disease. Given the mutation underway towards privatization of the veterinary services' delivery, it is likely that this trend will be extended beyond the present and until an almost "ideal" vaccine has been made available. Thus optimization of the use of antibiotics and a precise description of the circumstances under which they must be prescribed deserve due attention. This paper describes the rationale behind undertaking research activities aimed at answering some key questions related to the use of chemotherapy that were viewed as taboo as the subject was considered a closed one since the sixties.

1   Introduction

Spread of CBPP is influenced by closeness of contact, intensity of infection and the proportion of susceptible animals within the population under consideration. Control, so far, has been dependent on vaccination using live vaccines. This has been quite effective until the 1960s as National Veterinary Services were able to control animal movements, organize structured vaccination campaigns and could afford to pay for their cost.

Today, in most cases, organized government vaccination programmes are instituted months after the onset of an outbreak both because of the usually late recognition of new outbreaks and the length in mobilization of resources. In the meantime, whether the disease is suspected or not, livestock owners resort to treatment, generally with oxytetracycline or penicillin- streptomycin. They treat only sick animals and usually only once. They separate two groups and transfer any sick animal to the affected one where they treat it.

Although antibiotics could not be proposed to replace vaccines, it is interesting to compare these two tools from the standpoint of the beneficiaries, i.e: livestock owners (Table 1).

Table 1.   Comparison of chemotherapy and vaccination

	Chemotherapy	**Vaccination**
Expected results	rapid and spectacular	slow
Initiative of prescription	farmers, regulation not enforced, encouraged by veterinary auxiliaries, (privatization of veterinary services)	government, delays, respect of cold chain
Type of initiative	Reactive, (minimum number of injections)	Proactive

2   Known and assumed facts and figures

Mycoplasma mycoides subsp. *mycoides* (SC type) (*Mmm*SC), the agent of CBPP is susceptible to a range of antibiotics. Although dedicated studies are rather scanty, it has been shown *in vitro* that *Mmm*SC is susceptible, in decreasing order of importance, to Enrofloxacin, (fluoroquinolone), tylosin (timilcosin?), doxycyclin and lincomycin.

In vivo, peak blood concentration of enrofloxacin was 5·4 mg/ml at 2h following intravenous administration of the drug at a dose rate of 5mg/kg when it was administered to six mid-lactating cows. This demonstrates a good distribution of this quinolone in the organism. Other observations have shown in the past the efficacy of spiramycin in curing clinical cases (Provost 1974).

Resistance of *Mycoplasma bovis* to old tetracyclines (oxytetracyclines, chlortetracycline) was reported in 1993 by Terlaak; but so far no plasmidic resistance was evidenced either in *Mmm*SC nor in other mycoplasmas.

The EMPRES CBPP concept paper (1995) states that little research work has been carried out on the effect of antibiotics on the pathogenesis of the disease but it is likely that the sequence of events is as follows:

(a) Treatment during the incubation period (before clinical signs) — treatment should kill the causative organism and it may be that a single treatment is sufficient. The animal will recover but may not develop resistance to the disease nor develop a serological response.

(b) Treatment of clinical cases — early clinical cases may respond to prolonged treatment but advanced cases may not. In both cases, if the animal survives, it is possible but not certain that the treatment will result in a chronic carrier condition. The consensus is that such animals will not easily be detectable by serological test.

In other words chemotherapy may cause development of sequestra or carrier state in recovered animals which may become potential sources of spread of the disease; although this claim has never been substantiated through field experiments.

(c) Treatment of chronic case or "lungers" — the consensus of opinion is that treatment will have little effect on these cases as the causative organism, if present, is walled up in the sequestrum.

(d) Treatment of vaccinated animals — treatment in recently vaccinated animals will kill the vaccine strain and thus abort the development of immunity. Animals vaccinated sometime in the past will have little remaining resistance and can be regarded as susceptible animals.

The conclusions must be that treatment is only of value if cattle are in the incubation stage and is contraindicated if they have been recently vaccinated. It follows that treatment maybe of value in two particular situations:

- Where cattle are moving from infected areas into free areas (but only when the herd of origin is disease free). Some may be incubating the disease and treatment of all animals in the herd may abort the epidemic.
- An outbreak of disease where cattle are quarantined awaiting for slaughter. Some of the cattle will be advanced cases and will die, but others will be incubating the disease. In the normal course of the events some of these will eventually die and some will become "lungers". This sequence of events may be aborted by treating the cattle that are not clinical cases. This course of action may be of particular value in reducing the cost of an outbreak where the herd is destined for slaughter and the meat of the survivers has a salvage value.

Prescription of antibiotics should however be the responsibility of veterinarians only, and the withdrawal period should be respected in all cases.

3   Questions to be answered

3.1   Independent of treatment
- Is chronic carriage important in CBPP transmission and maintenance?
- Is formation of "sequestra" the only form of *Mmm*SC chronic carriage?
- What is the risk of transmission of CBPP from chronic carriers?
- What proportion of sequestra harbour infectious organisms and for how long?
- What happens in infected animals following vaccination?

3.2   Relating to treatment

Does treatment of an infected herd lead to production of a large number of chronic carriers ("lungers")? In other words, re. ATB causing perpetuation of chronicity in affected herds?

- How does it compare to vaccination?
- Are there modern chemotherapeutic agents and formulations, which have enhanced therapeutic value for CBPP compared to tetracyclines, spiramycin and tylosin? (such as Tiamulin, Tilmicosin and other new generation quinolones);
- Can a single injection of a suitable preparation result in sterile convalescence (as long-acting oxytetracycline preparations seem to operate in contagious caprine pleuropneumonia)?
- Can this injection abort infection during the incubation period?
- What effect does treatment have on circulating antibody levels?

- What is the benefit-cost of treating sick/in-contact animals destined for slaughter? (taking into account the problem of residues).

4   Current work at Sokoine University, Tanzania

Research will be conducted in the Usangu plains in the southern highlands of Tanzania where the disease is known to be rampant. Three naturally infected herds will be carefully identified (approximately 100 animals in each herd; high morbidity rate; high off take rate in order to enable examination of a sufficient number of carcasses, willingness to cooperate, etc.). Two of these herds will be left unvaccinated and one will be vaccinated in the week preceding the administration of antibiotics (to see the effect of pre-vaccination on the course of the disease among treated animals).

Each herd will constitute eight groups of animals. Four groups of clinically infected animals with six to eight individuals each will receive (double blind experiment):

Group C1: 2 oxytetracyclin injections, 14 days apart
Group C2: 1 injection of a quinolone derivative
Group C3: 1 injection of timilcosin
Group C4: placebo

Similarly, 4 groups (15 animals each) will be taken from apparently healthy, in-contact animals; they will be submitted to the same treatment regimen.

5   Research needs

Work should be done under laboratory conditions with experiment infection, comparing effect of different ATB and different regimens to: either identify one or more ATB/REG able to cure disease and infection, or other ATB/REG which prescription could be advocated under specific circumstances and without compromising vaccine based control strategies.

Search for resistance plasmids should also be conducted, although this is unlikely to succeed as plasmids sought for the purpose of studying the genome have not been found in Mycoplasmas so far.

6   Conclusion

Sensitive and specific immunodiagnostic tests (Bashiruddin *et al.,* 1994) and immunoprophylaxis, coupled with efficient slaughter policy of sero-positive animals are measures recommended for the control and eradication of CBPP (OIE, 1993). However, most CBPP-endemic countries of Africa are incapable of implementing the test and slaughter policy because of the huge financial costs involved. As a result, treatment of affected animals (usually at farmer's requests) due to rampant vaccine and vaccination failures are usually resorted to during outbreaks. This fact of life should be acknowledged and a thorough investigation on the role of chemotherapy as an adjunct to vaccination and quarantine in the control of CBPP should be given due attention from all concerned.

It is recommended that commercial pharmaceutical industry should sponsor appropriate research on the role of chemotherapy.

SURVEILLANCE AND CONTROL STRATEGIES: CBPP IN WEST AFRICA

M. Kané

1   Introduction

The improvement of the production and the productivity of animals is a sine quanon condition to reach food security in West Africa. Animal diseases are one of the major constraints at the development of animal productions. CBPP is now the most important disease of cattle in West Africa after the success obtained in the control of rinderpest in this region with PARC project. The disease is regularly recorded in most of the West African countries (Benin, Burkina Faso, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Mauritania, Niger, Nigeria and Togo). CBPP has not been reported in Gambia and Senegal for at least ten years. But their reinfection is possible because of transboundary movement of animals and the insufficient sanitary measures.

2   Geographical distribution

The situation of CBPP during the past five years in seven countries of West Africa is recorded in Table 1. It appears that with the exception of Senegal, CBPP was observed in indicated countries.

3   Factors of endemicity of CBPP

(i)   Weakness of sanitary measures of control.
(ii)   Transhumance.
(iii)   Concentation of animals round water sources.
(iv)   Cattle trade movement.
(v)   Temptation to antibiotherapy.

4   Strategies of control

(i)   Obligatory declaration of suspicions.
(ii)   Vaccination: annual vaccination campaigns are organized by most of the countries (Table 2).
(iii)   Control of movement.
(iv)   Meat inspection.
(v)   Identification, isolation and slaughtering of diseased animals.

5   Recommendations of Nouakchott workshop (February 1998)

(i)   The reactualization of trade, transhumance and nomadism routes.
(ii)   The execution in each country of epidemiosurveillance programme of CBPP.
(iii)   The execution of emergency programme in relation to EMPRES–FAO.
(iv)   The generalized massive and coordinated vaccination for at least 3 years.

(v)   The necessity to encourage training activities of veterinarians, livestock owners etc.

(vi) The necessity to continue research on CBPP particularly for validation of ELISA test and reinforcement of diagnostic capacities of national laboratories in collaboration with regional laboratories.

(vii) The support of Governments, OAU–IBAR, FAO and partners of development to African veterinary services and national diagnostic laboratories for the control and eradication of CBPP in West Africa.

Table 1. Evolution of CBPP in regions

Country		1993	1994	1995	1996	1997
Burkina Faso	F	4	8	3	8	8
Côte d'Ivoire	F	12	6	27	11	10
	Ma	2662	797	945	1018	1738
Guinea	F	60	30	28	26	22
Mali	F	19	21	11	12	15
	Ma	1362	975	695	150	591
	Mo	440	390	294	47	230
Mauritania	F	6	8	5	7	10
	Ma	6	8	7	123	54
	Mo	24	16	7	153	38
Niger	F	4	8	3	8	9
	Ma	12	23	24		
	Mo	5	35	13		
Senegal	F	0	0	0	0	0

F: Number of cases;
Ma: Number of sick animals;
Mo: Number of dead animals

Table 2. Cattle vaccinated from 1993 to 1997

Country	1993	1994	1995	1996
Burkina Faso	1173216	491921	834186	1481672
Côte d'Ivoire	993542	–	832146	732657
Guinea	459498	126285	410326	383511
Mali	1649206	1231091	1517668	1180518
Mauritania	–	–	–	–
Niger	502888	634659	344313	626456
Senegal	1152627	1203278	1192728	1190268

SURVEILLANCE AND CONTROL STRATEGIES: EAST AFRICA

Walter Masiga, Rene Bessin and Paul Rossiter

Summary

The countries of East Africa currently regard contagious bovine pleuropneumonia as the most important transboundary disease of livestock. In order to prepare an appropriate, coordinated, international control programme, increased surveillance for the disease is needed to generate accurate data on the incidence and distribution of the disease. At present surveillance for CBPP still relies on basic techniques such as abattoir surveys. These generate useful information if effectively carried out but there is still a need for better laboratory tests to detect infection in live animals.

Control in East Africa is based upon livestock movement control including quarantine and testing for infected animals, vaccination, and, in some circumstances, slaughter of infected stock. The priorities are to prevent any new infections of uninfected zones and eradication of the disease from infected high potential cattle populations. The incidence and distribution of CBPP in the endemic pastoral areas must also be reduced through the reintroduction of effective vaccination and movement control.

1 Introduction

The past decade has seen a significant increase in the distribution and incidence of contagious bovine pleuropneumonia (CBPP) in East Africa. All countries in the sub-region are infected and most report that this infection is now the major epidemic disease affecting their livestock. The Inter-African Bureau of Animal Resources of the Organization of African Unity (OAU–IBAR) recognizes this and that there is now a need to combat CBPP through coordinated control campaigns at a sub-regional and continental level. However, there is still a shortage of accurate data on the true incidence and distribution of the disease, both of which are needed in order to prepare a scientific and economically sound approach to better control. This data must be generated by surveillance.

2 Types of surveillance for CBPP

The main surveillance techniques used for CBPP in East Africa are: clinical diagnosis, pathological diagnosis including abattoir surveys, serology, and bacterial culture and identification.

2.1 Clinical diagnosis

Epidemics of CBPP in highly susceptible populations are diagnosed without difficulty on clinical grounds supported by epidemiological evidence such as the absence of routine vaccination, animal movement from infected areas etc. Similarly the disease is diagnosed clinically in the established endemic areas where there is little vaccination, and where it is usually well known to livestock owners.

2.2 Gross pathology

Typical gross pathological lesions in acutely affected cattle that die provide useful confirmation. Abattoir surveys can detect acute cases and also reveal chronic lung lesions including sequestra. They offer the easiest and least expensive method to survey for CBPP in East Africa at present. Limited abattoir surveys are being carried out in Tanzania, Uganda, Rwanda and Kenya but need to be expanded in order to assess the full national impact of the disease. In addition they need to be standardized so that results can be compared at a sub-regional and regional level.

2.3 Serology

The CFT is useful on quarantined cattle and herds that have not been vaccinated in the past three months. However, it is difficult to use as a surveillance tool on free-ranging pastoral herds though it has some value as a "herd" test under these circumstances. A serology network implemented by the IAEA/FAO Joint Division in Vienna is currently validating a new competition ELISA for detecting antibodies to CBPP. Initial results are encouraging in that the sensitivity and specificity of the cELISA are an improvement on the widely used CFT. This test also appears to detect antibodies only in infected rather than vaccinated animals. If true this would be a distinct advantage that would open up new opportunities for serological surveillance of CBPP.

At present, serological testing for antibodies, either with the CFT or the cELISA or both tests, can be carried out in Ethiopia, Kenya, Rwanda, Sudan, Tanzania and Uganda.

2.4 Mycoplasma culture

It is possible to culture *Mycoplasma mycoides* subsp. *mycoides* SC (*Mmm*SC) from the nasal secretions of infected cattle. However, it is too expensive and time consuming to be used as a routine surveillance tool for mass screening of cattle populations. In addition this method is less sensitive when used on samples such as nasal secretions collected from live infected cattle compared to its success on lung tissues from dead animals. At present it is used for laboratory confirmation of lung specimens from the field and from abattoir survey materials. Kenya, Tanzania, Ethiopia and Sudan have the technical capacity for microbial culture of *Mmm*SC.

3 The results of recent disease surveillance

Within the East African sub-region most countries are carrying out some surveillance for the disease mainly through their own national funds and initiative though some have a measure of external support. However, much of this work is still at an opportunistic or *ad-hoc* stage and there is a need for greater cohesion and reporting. Only one country in the sub-region, Uganda, has recently reported the results of surveillance carried out in abattoirs. The results from Kampala for the past 6 years are summarized in Table 1.

Table 1. The incidence of CBPP lesions detected in cattle lungs at Kampala slaughter - houses, and the number of CBPP vaccinations carried out annually from 1992 to 1998

Year	No. of cattle slaughtered	No. of cases of CBPP reported	% of slaughtered cattle with CBPP	No. of CBPP vaccinations administered annually
1992	14,277	335	2.350	1,504,718
1993	26,247	252	0.960	1,753,297
1994	56,970	77	0.140	911,742
1995	11,575	36	0.310	1,248,436
1996	25,538	32	0.130	620,709
1997	59,600	15	0.025	2,171,911**
1998 (to July)	12,133	3	0.025	–

** Data from August 1997 until end of April 1998

This shows a significant reduction, almost one hundredfold, in the incidence of CBPP being detected at the Kampala abattoirs, which tends to correlate with the last reported outbreak of clinical CBPP in June 1997 in Masindi District. This data from Kampala suggests that the CBPP situation in Uganda, especially in the centre and West of the Country is improving. However, the reasons for this are not certain. The vaccination returns suggest that no more than 50 percent of the country's cattle are immune to the disease. Further analysis of surveillance results and vaccination returns at the District level are in progress but can be misleading since slaughter stock often originate from other districts, or countries. On average, the incidence of CBPP being found in slaughter cattle in the districts in 1995 and 1996 was one to 2 percent.

These results are encouraging and show that Uganda has the basis on which to build a comprehensive surveillance programme for CBPP. They also confirm that abattoir surveillance must be carried out in conjunction with accurate information about the source of slaughter cattle. All deductions about changes in the incidence and distribution of the disease will be most useful when they can be correlated with field surveillance data from the source of the cattle and accurate information on control procedures including vaccination and treatment.

Serological surveillance with the CFT is being carried out in Kenya and Rwanda, and with the cELISA in Kenya and Uganda.

4   The difficulties with surveillance for CBPP

The situations in which surveillance and diagnosis are most difficult are populations in which there are variable amounts of vaccinal immunity, and the early stages of slowly evolving epidemics in extensively managed susceptible herds. These problems assume particular importance as vaccination is widely used in East Africa and because there is a need to recognize outbreaks of disease as early as possible.

Active surveillance for CBPP is useful but more difficult than, for instance, rinderpest. The clinical signs are less pathognomonic and laboratory confirmation in the live animal is not always possible. Currently a major problem facing surveillance in East Africa

is the inaccessibility of the large pastoral cattle herds throughout the sub-region. In part this is because the interface between these livestock owners and the animal health services has not been maintained and developed during the recent past. In addition, conventional state veterinary services are severely constrained financially and have difficulty operating. These issues can be corrected with improved funding and contemporary techniques for the delivery of animal health services, which are less dependent upon state intervention. In several countries it will also be necessary to have CBPP surveillance, including abattoir surveys, instituted as a routine function of the animal health delivery systems if reliable data is to be obtained. This must be supported by accurate, affordable and simple laboratory confirmation techniques.

Research into surveillance for CBPP is also still needed. Better tests to confirm disease in live animals are essential. Increased knowledge about the sensitivity and specificity of surveillance methods would be useful. For instance, are abattoir survey findings proportional to the prevalence of disease in the field? Can they be used to help map out hyper-endemic as opposed to sporadically infected populations?

5   PARC policy for surveillance and control

Past experience in many parts of the world has shown that CBPP is an eradicable disease. However, the same experience has also shown that this cannot be done as easily as with rinderpest. Therefore, although eradication must be our long-term goal for CBPP in Africa, effective control is our target in the short to mid-term.

The first priority is to ensure that there are no further outbreaks from the endemic areas into neighbouring disease-free territories as occurred in Rwanda and Tanzania. This will require improved control of livestock movement especially at borders and along international trade routes; and improved surveillance/vigilance in the countries at risk in order to ensure early detection of any new outbreaks. Its sustainability will also depend upon improved control in the endemic areas to reduce the risk of disease. It is very important that the endemic focus now established in Tanzania does not merge with endemic focus in Angola.

The second priority is to assess which areas of the sub-region can confidently be said to be sporadically as opposed to endemically infected. This will require accurate surveillance. The aim would then be to re-establish the sporadically infected areas as CBPP-free zones in which the disease occurs rarely if at all. Having re-established the status quo of a decade ago the main thrust would then turn to CBPP in the endemic pastoral areas. This will be a major undertaking necessitating educating and persuading the livestock owner and trader to accept the re-introduction of some degree of movement control; large-scale and, therefore, expensive, mass vaccination campaigns to induce high levels of herd immunity, quarantines and continuous surveillance. Clearly these can all take place simultaneously but the goals would be to progressively restrict the infection back to its main endemic foci as has been achieved with rinderpest.

In the long term, eradication is the goal. This is close to being achieved for rinderpest. In this regard, the OIE "Pathway" is proving a valuable tool as countries move through the final stages of eradication. A similar pathway has been prepared for CBPP. It will prove beneficial if we develop control strategies that allow us to use this pathway to

guide countries and clusters of countries towards improved control in such a way that the process leads smoothly into final eradication.

OAU–IBAR believes that the control of a transboundary disease such as CBPP can only be successful when the efforts of individual countries are harmonized through a coordinated international programme, such as PARC. These programmes provide conformity and standardization of technical and managerial inputs, and cohesion of control operations across international borders. This ensured that within the overall campaign all countries paid sufficient attention to important issues such as training of staff and sensitization of the livestock owning public. A coordinated campaign also sets standards and targets for disease surveillance and reporting, and monitors the performance of these.

OAU–IBAR and SADDC have jointly prepared a proposal for a project to eradicate CBPP in Southern Africa within which one East African country, Tanzania, is included. However, in East Africa as a whole there is no regionally coordinated surveillance programme for CBPP at present.

6   Conclusion

Several of the epidemic extensions of CBPP in East Africa in the last decade were due, at least in part, to a failure to maintain accurate surveillance on this disease. Thus good control is impossible without adequate surveillance in the sub-region. OAU–IBAR recognizes this and is preparing to include surveillance for CBPP within its regional epidemiology network established for rinderpest. Through the network it should be possible to encourage and standardize CBPP surveillance throughout Africa leading to an accurate epidemiological assessment of its true incidence and distribution. This will provide firm evidence upon which to assess disease risk and develop a cohesive programme of continental control.

CBPP SURVEILLANCE AND CONTROL STRATEGIES: SOUTHERN AFRICA SUBREGION

P. G. Sinyangwe

1   Introduction

Zambia has experienced three contagious bovine pleuropnemonia (CBPP) outbreaks since the beginning of the century. All these have been recorded in the Western province of Zambia. The outbreaks in Western province have been of Angolan origin. Two Complement Fixation Test (CFT) positive cases were recorded in the Northern province of Zambia in February 1998. CBPP is classified as a disease of national economic importance in Zambia. The control of the disease is the responsibility of the Government with the cooperation of the farming communities.

1.1   The first outbreak (1915 to 1944)

The first recorded outbreak of CBPP occurred in 1915 near Lealui as a result of Barotse oxen involved in the Angolan–Portuguese boundary commission which mixed with oxen from Angola. The oxen returned to their kraals incubating the disease and within a year the disease had spread throughout the Barotse Protectorate. By 1926, 280 000 cattle had died as a result of the disease.

Control measures instituted at that time included vaccinations; compulsory sale for slaughter of clinically affected animals, the establishment of a cordon and an enforced cattle-free buffer zone along the Angola/Zambia border.

1.2   The second outbreak (1969 to 1973)

The disease was first suspected in 1969. It is presumed that the disease was brought about by a combination of factors notably the Angolan refugees entering Zambia with their cattle from 1966 due to political pressure, dismantling of the cordon line and the increase in trade and cattle movements between Zambia and Angola.

After the disease was confirmed by laboratory tests, a control zone 30 km wide from the Angola/Zambia border was established extending from the South Lueti river to Sinjembela. All the 13 468 cattle resident in this zone were destined for slaughter when it was realized that the disease had spread outside the control zone.

The whole province was quarantined in November 1970 and a permanent cordon line and buffer zone were established. Mass vaccinations were commenced in February 1971 using the lyophilized T_1 vaccine. An eradication campaign was launched in 1972 once the disease had been reduced to manageable levels by vaccinations. This involved testing of all animals in areas where the disease had occurred and slaughtering with compensation of all the reactors. These measures successfully brought the disease under control. Surveillance in the buffer zone detected sporadic individual cases in 1986, 1988, and 1990 which did not result in the disease becoming established.

1.3   The third outbreak

A combination of control measures kept Zambia free from CBPP for almost 25 years. However, towards the end of 1995 Veterinary field staff reported increased resistance amongst farmers to have their animals vaccinated and branded. This was linked to the closure of the main abattoir in the province situated in Mongu. Farmers reasoned that it would be easier to illegally market their animals on the rail line if they did not bear the vaccination brand marks. The signing of the peace accord between the Government in Angola and UNITA resulted in increased cross border movement of people and their cattle. Based on these concerns a CBPP mission from Headquarters visited Western province towards the end of 1996. This mission's findings confirmed the existence of an imminent threat of CBPP.

Investigations for CBPP suspected cases on the Angolan/Zambia border had been going since October 1996 without the disease being confirmed. In April 1997 CBPP was confirmed based on post mortem findings and laboratory tests in Sinjembela area outside the buffer zone.

2   Control strategies

Drawing from the past and present experiences several control measures were effected to contain this (1997) outbreak. These included:

> *Formation of disease risk zones:* This was based on proximity to the outbreak areas and the likely cattle movements. The risk zones were classified as follows:
>
> - Focal area where the disease actually broke out;
> - Primary risk area — the area west of the Zambezi river in Senanga and Sesheke districts;
> - Secondary risk area — the area next to the primary area which includes Senanga east, Sesheke east, Mongu and Kalabo districts;
> - Tertiary risk area — the rest of the province.
>
> *Livestock movement restrictions:* to prevent movements from the province and between districts within the province; in the focal area no mixing of herds and no animals to leave the primary risk area.
>
> *Serological testing:* immediate testing prior to vaccination of all herds in the high risk areas with post mortem examination of positive reactors, 3 264 animals were tested in the first round of which 76 were positive. About 6 479 animals were tested in the second round of which eight were sero-positive.
>
> *Vaccination against CBPP:* using a quality-assured monovalent CBPP vaccine in the focal, primary and buffer zone, 145,317 animals were vaccinated in the first round and 144 353 were vaccinated in the second round.
>
> *Slaughter of entire herds:* where the disease was confirmed on post-mortem with compensation of the owner 1 050 cattle were slaughtered with compensation. CBPP surveillance at abattoirs and slaughter slabs.

3 Surveillance

In the Western Province CBPP surveillance has been going on since the second outbreak (1969 to 1973). However, there was laxity in the late 1980s due to the fact that there were no more active cases of CBPP and consequently funding towards CBPP control was reduced. The cordon line operations were very instrumental in CBPP surveillance with a fully manned cordon line and motorized patrols by the cordon base officers at Shangombo and Sikongo.

One of the shortcomings is the current restructuring exercise which has left the cordon line manned at 40 percent capacity. The cordon line itself though requires attention not fully operational with the fence not existing and the track not cleared for kilometres.

After the second round of CBPP control activities (Nov/Dec) a surveillance station was established at Kaanja in the primary risk area. This station is manned by a Veterinary Officer assisted by a Livestock Officer. This station is charged with the responsibility of:

- Carrying out monthly visits to the A7 (surveillance) herds. These are the herds where serological positives were recorded during the second round of CBPP testing. However, these herds were not slaughtered because the reactors showed no post-mortem lesions for the disease.
- Patrolling the focal area monthly.
- Supervising cattle disease control guards employed to ensure that there are no illegal cattle movements into and out of the focal area including movements from Angola.
- Issuing stock movement permits and deal with any illegal movements.
- Carrying out extension meetings with the farmers on CBPP and its control measures using an NGO/Keepers Zambia Foundation.
- Patrolling the cordon line ensuring that activities are being carried out.
- Using the Veterinary Inspectorate to continuously assess the disease situation.
- Collecting CBPP samples for diagnosis from suspected cases.

The FAO TCP assists in meeting some of the costs of running the surveillance programme. However, most of the equipment is yet to be procured.

In the Northern Province, surveillance commenced in 1995 and was intensified this year when a serological survey of 20 percent of cattle in the surveillance zone was carried out. Further sampling will be done later this year. The abattoir at Nakonde and other border areas are regularly supervised to detect evidence of lesions. Extension meetings in the border areas are carried out to sensitize farmers on CBPP and the need to control it.

4 Conclusion

Concerted efforts have eliminated two incursions of CBPP into western Zambia in the past. The control measures implemented kept Zambia free of CBPP until it was re-introduced in 1997. The control measures initiated after the 1997 outbreak appear, once again, to have brought the disease under control, although continued surveillance is necessary to maintain this. However the 1997 outbreak and the reintroduction of disease that occurred in 1998 highlight the fact that, while the disease remains endemic in Angola, western Zambia will always be at risk.

In the Northern province the disease is confined to the border areas in herds that graze well into Tanzania. Without continued movement control, vaccination and surveillance, the risk of the disease spreading further into Zambia exists.

Long-term control is, however, dependent on regional collaboration between the countries which are affected by the disease and those threatened by it. In the southern African region it is strongly felt that the following areas would be beneficial in the region:

- Regional collaboration in CBPP control with the harmonization of vaccination campaigns between countries.
- Standardize diagnostic procedures in the region.
- Strengthen quarantine and movement regulations.
- Establish a regional reference laboratory and a regional research database.
- Encourage exchange of scientific information and materials.

CBPP SURVEILLANCE AND CONTROL STRATEGIES IN BOTSWANA

K. V. Masupu

Summary

Resurgence of contagious bovine pleuropneumonia (CBPP) in Botswana in 1995, after a period of over fifty years of freedom from it, and its surveillance and control strategies are reported. The main vehicle of spread under Botswana conditions has been identified to be movement of cattle. Measures adopted to eradicate it comprised of zoning, creation of physical barriers in the form of fencing of the affected area, total cattle depopulation and intensive surveillance.

1   Introduction

Botswana has been free from contagious bovine pleuropneumonia (CBPP) since the disease was eradicated in 1939. In February 1995, CBPP occurred in the Xaudumo Valley, Ngamiland district at the extreme north-western part of Botswana. Xaudumo Valley is adjacent to the Kavango district of Namibia where CBPP is known to occur (Schneider *et al.,* 1994). This paper presents the CBPP outbreak in terms of the situation in Botswana and the measures (zoning, creation of physical barriers in the form of fencing of the affected area, total cattle depopulation and intensive surveillance) taken to eradicate it.

2   Zoning

The immediate intervention to contain the disease from spreading further was to divide the affected area into red, yellow and green zones, representing the infected, possibly infected or suspicious and non-infected or clean zones respectively. These zones were separated by picketers and later cordon fences were placed at strategic points to restrict cattle movement.

3   Control strategy

The strategy adopted was to eradicate CBPP by slaughtering all animals in the infected zone and salvage their meat. This necessitated putting up quarantine infrastructure. While infrastructure was being constructed, all clinically sick cattle in the red zone were destroyed in an effort to reduce infection pressure. The apparently healthy ones (13 000) were vaccinated with T_1SR CBPP vaccine produced locally by the Botswana Vaccine Institute (BVI) and identified by a cervical "**I**" brand. Those in the adjacent Yellow zone (12 000) were treated the same and identified with a cervical "**Y**" brand.

The number of cases did not decrease in the Red zone, and by June 1995 clinical cases were observed in the Yellow zone, indicating that the disease was spreading despite the measures being taken. Thus, vaccination was stopped immediately and a decision made to eradicate the disease by total depopulation.

4   Surveillance

It was very important that the country institutes an intensive surveillance programme for contagious bovine pleuropneumonia (CBPP) in the affected zones and adjacent ones. The following CBPP surveillance components were put in place: *routine clinical inspection of herds, CBPP sero-survey* in the high risk areas twice a year, carrying out *necropsy* of all dead cattle that were known paying particular attention to the *lungs;* all slaughter slabs and butcheries were assigned a technical officer, creating *public awareness* through posters, CBPP booklets distribution and showing film.

Seromonitoring started in August 1995 to date, and hence between October 1995 and May 1996, blood samples were collected from 78 917 cattle belonging to 2142 kraals (family herds). Sampling was not randomized but every animal was sampled and bled (i.e. census sample). Sera were tested for *Mycoplasma mycoides* subsp. *mycoides* (small colony) (*Mmm*SC) complement fixing antibodies using Complement Fixation Test (CFT) (Campbell and Turner 1953) at the National Veterinary Laboratory (NVL) in Gaborone. Results were reported as either positive or negative.

Statistical analyses were performed using BMDP statistical software (Dixon, 1990). Specifically the BMDP2D programme was used for detailed data description, and BMDP4F to perform trend analysis (Pearson Chi–square) in the proportions of CBPP seropositive herds with respect to month of testing. BMDP3D (students t–statistics) was used to test the difference in herd sizes between the positive and negative herds.

Currently CBPP serosurveillance is being undertaken in a statistically selected sample in high risk disease control zones of 1 (Chobe), 2a (Okavango) and slaughter slabs, butcheries and abattoir surveillance.

Ngamiland restocking began in April 1997 and finished in March 1998. Cattle were transported very long distances and were acquired throughout the country. This was identified as a hazard for disease introduction and every effort was made to avert the danger of disease through quarantine, clinical inspection and testing. Diseases tested were CBPP, FMD and Brucellosis.

All cattle for restocking were sampled for CBPP and quarantined (21 days) on entry into Ngamiland. There after those in disease control zones 1 and 2a are examined and sampled for CBPP twice a year. Those cattle in zone 2a were introduced in December 1997; they were sampled in March 1998 and are due to be sampled for the third time in October/November 1998. So far we have never had a CBPP positive (or suspect) in all the 70 000 cattle.

Since April 1997, 122 cattle have been necropsied in disease control zones 4a (Boteti), 1 (Chobe) and 2 a,b,c,d (Ngamiland). Fifty-one (51) animals were from Boteti, 4 from Chobe and 67 from Ngamiland. A special form has been designed for use by the field and abattoirs in inspecting the lungs. Causes of death were attributed to plant poisoning and pasturellosis as confirmed by field and laboratory. Three cases had gross lesions similar to those of CBPP but lung samples submitted yielded only pastuerella organisms on culture.

5   Conclusion

Botswana remains free of CBPP but the threat remains as it was in 1994. There is therefore a need for regional collaboration to eradicate CBPP out of Southern African region and the rest of the continent.

THE OIE PATHWAY

A. Provost

Summary

"The initial draft of this document was prepared in June 1993 by the Ad hoc Group on Contagious Bovine Pleuropneumonia Surveillance Systems of the Office International des Epizooties. That draft was then amended in February by the Group of experts convened at the request of the International Committee to examine issues relating to the surveillance of the disease, with particular reference to the effects of vaccination programmes on surveillance systems. This updated report includes the following:

- *epidemiological and other factors which influence the choice of surveillance systems*
- *sampling and surveillance strategies*
- *diagnostic methods applicable to surveillance systems*
- *the repercussions of vaccination on surveillance systems."*

For the complete text, please refer to:

Recommended standards for epidemiological surveillance systems for contagious bovine pleuropneumonia. *Rev. sci. tech. Off. int. Epiz.* 1977. **16**: 898-904.

FAO GUIDELINES FOR EFFECTIVE PREVENTION AND PROGRESSIVE CONTROL/ERADICATION OF CBPP IN AFRICA

A. Benkirane

As with all epizootic diseases the essence of CBPP control, given early recognition, is rapid response to reduce the weight of infection available for transmission and to eradicate the infection in the shortest possible time so that the risk of transmission is minimized or, preferably, eliminated. This is most effectively achieved with CBPP by slaughter of all infected cattle and those exposed by contact, but to be feasible, this requires early recognition of primary outbreaks through effective surveillance and rapid response facilitated by an effective and practiced contingency plan. These conditions might not be met and, pragmatically, this approach might not be feasible;

alternative means = vaccination and quarantine

To be effective vaccination must cover 100 percent of cattle within a readily, epidemiologically and geographically definable large area, thus involving large numbers of cattle. Vaccination must be repeated, initially at short intervals and thereafter annually over several years, i.e. not less than three to five years. Such vaccination must be maintained until evidence of CBPP eradication is demonstrated by structured surveillance as is being proposed by the OIE. Thus intensive immunisation requires sustained efforts over several years; this might prove difficult to achieve.

EMPRES Concept Paper on the control of CBPP in Africa with special reference to Eastern and Southern Africa

Basically, the concept envisages the division of the region into three epidemiological categories:

- the free areas where emphasis has to be placed on enhanced surveillance and CBPP emergency preparedness;
- the infected areas where intensified control action needs to be instituted relying on either a slaughter policy or a prolonged quarantine and systematic vaccination policy; and,
- the cordon sanitaire sufficiently defined to separate clean and infected areas.

This cordon is envisaged to be in two parts:
- an Eastern buffer zone covering the international borders between Tanzania and Zambia, Malawi, Mozambique, and the area of DRC immediately to the west of Lake Tanganyika (Ituri, Nord and Sud Kivu);
- a western buffer zone stretching from the Atlantic Coast across northern Namibia, northern Botswana and western Zambia.

These buffer zones will have two components:

(a) a surveillance zone at least 50 km deep covering the disease-free side of the international border in which no vaccination will take place while intensive surveillance and animal movement control will be enforced; and,

(b) a control zone at least 100 km deep covering the infected side of the border immediately adjoining the surveillance zone. Control will be maintained in this area by intensive vaccination, surveillance and movement control.

Zoning should also be devised within the control zone:
- Infected zones (active foci) where quarantine must be strict and coupled with vaccinations at short intervals. OAU/OIE/FAO guidelines recommend vaccination at months 0, 3, 9, 21, then annually.
- High risk zone with active disease search (surveillance) and vaccination at months 0, 6, 18, then annually.
- Low risk zone. Surveillance and annual vaccination.
- Minimal risk. No vaccination; early warning.

West Africa

A draft strategy was devised in Nouakchott, Mauritania, in February 1998 for a sub-region comprising 15 countries (stretching from the Atlantic Ocean to the Niger/Nigeria — Chad/Cameroon borders).

The sub-region was divided in three groups of countries :

Group 1:

Countries not declaring CBPP (Senegal and Gambia) should enter the CBPP OIE pathway after:

- ceasing vaccination (last campaign this year) and declare themselves provisionally free of CBPP.
- A cordon sanitaire (buffer zone) has to be established on the border of Senegal with other neighbouring countries. It will comprise two zones:

(1) Surveillance zone, 50 km wide where no free vaccination should take place.

Should CBPP break through in this zone, the whole affected herd(s) should be destroyed and their owners compensated. The source of infection must be determined and contact herds examined for CBPP. If other herds are found infected the same should apply unless it becomes obvious that such a strategy is no more viable. The surveillance should then be transformed into control zone and a new surveillance zone ought to be delineated.

(2) Control zone, 100 km deep, where mass vaccination is implemented annually during at least 5 years (free vaccination encouraged).

Group 2:

At risk countries (recipients of infection): mostly countries on the coast (Nigeria, Benin, Togo, Ghana, Côte d'Ivoire, Liberia, Western Guinea, Sierra Leone and Mauritania)

Vaccination to be instituted compulsorily upon departure in transhumance. Vaccinated animals to be eartagged.

In case of apparition of new outbreaks, the whole population at risk should be re-revaccinated, at governments' expense. The OIE/FAO/OAU–IBAR recommendation should be adhered to i.e vaccination at 0, 3, 9 months then annually.

Also encourage slaughter with compensation.

It is hoped that in the near future, the use of cELISA will enable to reconsider this constraining scheme.

Group 3:

(Endemic countries) Mali, Guinea, Eastern Guinea Bissau, Burkina Faso and Niger.

A secular endemic infection area is present in the delta of central Niger where animals coming from Mali, Burkina Faso, Niger and Mauritania gather frequently.

Another seems to cover East Guinea and Guinea Bissau.

Also South-west of Lake Chad (Nigeria, North-west Cameroon and Chad) is endemic thus this strategy is to be extended to other countries on West and Central Africa.

Vaccination must be compulsory, generalized, coordinated and repeated at least over 5 years.
Free of charge?
Yes, if sanctions vis–à–vis non-respect is foreseeable.
Also comitment is required from the part of veterinary services to act massively in these endemic areas.

For groups 2 and 3, after 5 years, States should embark and commit themselves to the OIE pathway towards CBPP *eradication*.

Definition of *zones* (according to FAO terminology) is to be done during the transition period during which vaccination campaigns are carried out.

**FIRST MEETING OF THE FAO/OIE/OAU–IBAR CONSULTATIVE GROUP ON
CONTAGIOUS BOVINE PLEUROPNEUMONIA
5-7 October 1998**

LIST OF PARTICIPANTS

J. Bashiruddin
FAO Consultant
338 Aberdeen Street   Tel: (to be supplied)
Geelong West 3218   Fax: (to be supplied)
AUSTRALIA   E-mail: (to be supplied)

M.H. Jeggo
Head, Animal Production & Health Section
Joint FAO/IAEA Division
P.O. Box 100
Wagramerstrasse 5   Tel: (43) 1 2600 26053
A-1400 Vienna   Fax: (43) 1 26007
AUSTRIA   E-mail   M.H.Jeggo@iaea.org

M. Kané
Chef de la Division de santé animale
Direction nationale de l'élevage
BP 265   Tel: (223) 222 022 / 231227
Bamako   Fax: (223) 231 217
MALI   E-mail radiscon.bamako@malinet.ml

J. Litamoi
PANVAC Consultant for GCP/RAF/318
c/o FAOR in Ethiopia
P.O. Box 5536   Tel: (251) 1 338 001
Addis Ababa   Fax: (251) 1 338 844
ETHIOPIA   E-mail: panvac@telecom.net.et

J.B. March
Moredun Research Institute
International Research Centre
Pentland Science Park
Bush Loan, Penicuik EH26 OPZ
Edinburgh   Tel: (44) 131 445 5111
Scotland   Fax: (44) 131 445 6235
UNITED KINGDOM   E-mail: marcj@mri.sari.ac.uk

W. Masiga
Director, OAU–IBAR
P.O. Box 30786   Tel: (254) 2 338-544 / 334550
Nairobi   Fax: (254) 2 332-046
KENYA   E-mail
parcepid@users.africaonline.co.ke

K. Masupu
Deputy Director, Department of Animal
   Health & Production
Ministry of Agriculture
Botswana Government
P/Bag 0032   Tel: (267) 350 500
Gaborone   Fax: (267) 303 744
BOTSWANA   E-mail: masupu@info.bw

B. Morein
Head, Department of Virology
National Veterinary Institute
Veterinary Faculty
Swedish University of Agricultural Sciences
BMC Box 585   Tel: (46) 18 471 4571
S–751 23 Uppsala   Fax: (46) 18 504 603
SWEDEN   E-mail: Bror.Morein@sva.se

R. Nicholas
Central Veterinary Laboratory
New Haw, Addlestone   Tel: (44) 1932 357379
Surrey KT15 3NB   Fax: (44) 1932 354929
United Kingdom   E-mail: Nicholas@vla.maff.gov.uk

A. Provost
OIE Consultant
2, rue Fontaine   Tel: (33) 2 376 47175
Ezy sur Eure 27530   Fax: (33) 2 376 46993
FRANCE   E-mail: aprovost@minitel.net

J. Regalla
Laboratorio Nacional de Veterinaria
Estrada de Benfica, 701
P-1500 Lisboa   Tel: (351) 1 716 2075
PORTUGAL   Fax: (351) 1 716 3964

P. Rossiter
CTA – GCP/RAF/317
c/o FAOR in Kenya
P.O. Box 30470   Tel: (254) 2 725 069
Nairobi   Fax: (254) 2 727 584
KENYA   E-
mail:parcepid@users.africaonline.co.ke

F.G. Santini
Istituto Zooprofilattico Sperimentale
    dell'Abruzzo e del Molise
Via Campo Boario   Tel: (39) 0861 332234
64100 Teramo   Fax: (39) 0861 332251
ITALY

J. Sinyangwe
Director
Research and Specialised Services
Ministry of Agriculture   Tel: (260) 1 250274
Lusaka   Fax: (260) 1 236283
ZAMBIA   E-mail: cvri@zamnet.zm

F. Thiaucourt
CIRAD-EMVT, PATHOTROP
B.P. 5035
34032 Montpellier   Tel: (33) 4 675 93723
Cedex 1   Fax: (33) 4 675 93798
FRANCE   E-mail: thiaucourt@cirad.fr

FAO SECRETARIAT

Yves Cheneau
Chief, Animal Health Service, AGAH
Tel: (39) 06570 53531
E-mail: yves.cheneau@fao.org

Mark Rweyemamu
Senior Officer (Infectious Diseases/EMPRES), AGAH
Tel: (39) 06570 65772
E-mail: mark.rweyemamu@fao.org

Abdelali Benkirane
Animal Health Officer (Bacteriology), AGAH
Tel: (39) 06570 52681
E-mail: abdelali.benkirane@fao.org

Peter Roeder
Animal Health Officer (Infectious Disease Emergencies), AGAH
Tel: (39) 06570 54637
E-mail: peter.roeder@fao.org

Roger Paskin
Animal Health Officer (Epidemiology), AGAH
Tel: (39) 06570 54747
E-mail: roger.paskin@fao.org

Joachim Otte
Senior Officer (Veterinary Services), AGAH
Tel: (39) 06570 53634
E-mail: joachim.otte@fao.org

Ms. Egiziana Fragiotta
Clerk-Stenographer, AGAH
Tel: (39) 06570 52637
E-mail: egiziana.fragiotta@fao.org

FAO, AGAH Fax: (39) 06 570 53500
                   (39) 06 570 53023
                   (39) 06 570 55749

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GROUP PHOTO

Lower row **(from right to left): K. Ben Jebara; T. Obi; B. Morein; W. Masiga;**
A. Provost; Y. Cheneau; F. Santini
*Second row***: J. Otte; A. Benkirane; F. Thiaucourt, M. Kané; M.M. Rweyemamu;**
Ms A. Gervelmeyer.
*Upper rows***: J. March; M. Jeggo; D. Nyakahuma; J. Bashiruddin; J. Litamoi,**
P. Rossiter; P. Sinyangwe; R. Nicholas; J. Regalla.