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MALI ANIMAL PRODUCTIVITY & EXPORT PROJECT (APEX)

REPORT OF A CONSULTANCY
MISSION TO THE CENTRAL VETERINARY LABORATORY
BAMAKO, MALI

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A.1. RESUME DES TACHES EXECUTEES (EXECUTIVE SUMMARY - FRENCH)

Trois (3) banques de semences de reférence de la Péripneumonie Contagieuse Bovine ont été faites avec des souches de T1SR,de KH3J et de T1 44 de Mycoplasma mycoides variété mycoides. Elles ont été testées pour la stérilité, la numération, et l'inocuité sur des animaux de laboratoire.

Avec la souche T1SR, des lots de vaccin contre la Péripneumonie Contagieuse Bovine ont été préparés et soumis à une évaluation du vide, de la stérilité, de l'identité et de la numération. Tous les tests se sont avérés satisfaisants. Au cours de la préparation de ces lots de vaccin, certaines additions ou modifications dans le processus de fabrication, employé jusque là au LCV, furent introduites. Ces changements ont conduit à l'amélioration de la quantité et de la qualité du vaccin.

Un programme de formation sur place du personnel des Sections Production de vaccin contre la PPCB et du contrôle de la qualité a été établi et réalisé. La formation portait sur les différentes techniques utilisées dans la préparation et le contrôle de la qualité du vaccin contre la PPCB.Il a été ensuite permis au personnel de repéter les procédures de fabrication et de contrôle du vaccin sous la supervision du consultant. En outre, un protocole des méthodes à suivre est inclus dans ce rapport comme reférence pour le personnel.

A la suite des discutions avec le personnel du LCV à propos des recentes réactions post-vaccinales à la suite de la vaccination contre la Péripneumonie Contagieuse Bovine dans certaines zones du Mali, il semble tout à fait que le vaccin utilisé ait été fait avec la souche T1 44 qui a pu être administré aux animaux par une voie non recommandée pour les races bovines très sensibles. L'image clinique décrite était compatible avec les réactions post-vaccinales dûes à l'utilisation incorrecte de la T1 44.

A.2. RESUME DES RECOMMANDATIONS

1. * Au vu des faits préalablement établis, les banques de semences du vaccin T1 du LCV se sont revélées non homogènes en ce qui concerne le titre. Ces banques de semences doivent être détruites.

   Le LCV doit alors préparer les futures vaccins contre la PPCB en utilisant les banques de semences qui ont été préparées durant la mission du consultant. Il est ensuite recommandé que, vu les imprévisibles reactions adverses de la T1 44 (particulièrement par la mauvaise administration) et la durée relativement très courte de l'umminité coférée par la souche KH3J, le LCV doit fabriquer le dit vaccin uniquement à partir de la souche T1SR. Les banques de semences doivent être stokées à une température de -20 °c au plus et un recensement exact d'usage doit être fait.

2. * Pour faciliter le contrôle du pH de la culture vaccinale de mycoplasmes, l'indicateur , le rouge de phénol doit être ajouté au milieu de culture vaccinale.

3. * Comme le serum importé, le serum local de cheval ou d'âne supporte bien la croissance des mycoplasmes. Le LCV peut donc utiliser aisément le serum provenant de ses troupeaux d'ânes et de chevaux sans pour autant compromettre la qualité et la quantité des vaccins produits. Pour que la valeur nutritive du serum se maintienne, il doit être correctement traité et conservé.

   L'utilisation du serum local doit aboutir à une épargne considérable des coûts d'importation des

milieux de culture pour mycoplasmes.

4. * Pour minimiser les grandes variations entre les lots de vaccin, la recolte des différentes cultures doit se faire après addition du cotenu de différents ballons de production avant la repartition et la lyophilisation. Pour cela, un grand récipient de 30 litres de capacité doit être acquis. L'inconditionnel avantage de l'addition des cultures à la recolte est que la taille des cultures augmente tandis que le prix du contrôle de qualité est reduit.

5 * La pratique de diluer le vaccin contre la PPCB à la dose de reconstitution sur le terrain avant le titrage n'est pas une bonne pratique, car elle abouti à une sous estimation du comptage des mycoplasmes. Il est recommandé d'utiliser la pratique qui consiste à reconstituer le flacon dans 10 ml au maximum de diluant et les stades suivants de dilution doivent entraîner un changement de pipettes ou d'embouts de pipettes entre les dillutions successives sans pour autant toucher la surface du diluant avec les embouts ou les pipettes.

6. * Chaque flacon de vaccin contre la PPCB doit être testé pour le vide avant d'être utilisé. Le système courament utilisé (absorption de la solution de dillution par le flacon) ne peut être utilisé pour des raisons évidentes (il ne restera plus de vaccin). Il est donc nécessaire pour le LCV d'utiliser de façon routinier le testeur de vide deja existant pour chaque flacon de vaccin.

7. * Bien que la salle de repartition soit convenablement lavée et fumiguée avant utilisation, on ne peut pas compter sur la hotte sous laquelle se fait la repartition pour garantir la stérilité du vaccin manipulé. IL est donc nécessaire à ce que soit commandée une laye hotte à flou luminaire classe 2 afin que le vaccin soit reparti dans des conditions pouvant minimiser les chances de contamination.

8. * L'autoclavage de la suspension de stabilisateur (lait écrémé) à 36%, aboutit à une forte caramélisation. Il est par conséquent recommandé que le lait écrémé à utiliser pour la lyophilisation soit préparé à 20-25% et mélangé, et additionné au vaccin à recolter, sa concentration finale dans le vaccin recolté doit atteindre 4-5% .

9. * Pour que le processus de fabrication ne soit pas intérrompu par des pannes de machines (comme il a été remarqué au cours de cette periode de consultation) il est souhaitable pour le LCV d'acquerir un lyophilisateur additionnel. Le cycle de lyophilisation utilisé pour les lots actuels de souches et de vaccin peut être adopté parce-qu'il a fait ses preuves malgré le disfonctionnement de la machine.

10. * Les flacons en verre et d'autres recipients utilisés pour les cultures de mycoplasmes doivent être convenablement rincés dans de l'eau distillée purifiée. Les rincer uniquement dans de l'eau de robinet n'est pas suffisant parce-que certains produits chimiques utilisés dans le traitement de l'eau (ex. eau de javel) sont extrêmement néfastes pour les mycoplasmes. Pour cette raison le LCV doit compter sur une source adéquate de supplémentation en eau distillée pure. Le distillateur d'eau deja existant au LCV doit être absolument fonctionnel.

11. * De temps en temps, le LCV doit organiser, pour le personnel de la production du vaccin contre la PPCB et du contrôle de qualité, des visites et des formations dans des Laboratoires de référence en matière de production de ce vaccin en vu de leurs permettre d'acquerir les nouvelles techniques, technologies, et méthodes en la matière.

Par ailleurs, des séances de recyclage pouront être régulièrement organisées à l'intention de

ce même personnel pour lui permettre de corriger au fur et à mesure les erreurs éventuelles qui se glisseraient dans le processus de production du vaccin.

12. * Il serait souhaitable d'acquerir l'équipement additionnel mentionné en annexe de ce rapport pour les sections de fabrication du vaccin T1 et de contrôle de qualité afin de minimiser les chances possibles de contamination, pouvant survenir aucours des déplacements d'une section à une autre, au sein même du LCV.

B. EXECUTIVE SUMMARY

Three Contagious Bovine Pleuropneumonia (CBPP) Vaccine reference seed banks were established. The seed banks represent the T1SR, KH3J and T1/44 strains of *M. mycoides* subsp. *mycoides* (SC, bovine biotype). Prepared seed lot banks were tested for sterility, identity, viable mycoplasma content and innocuity in laboratory animals.

Using the established T1SR seed strain, batches of CBPP Vaccine were prepared and subjected to quality assessment tests for vacuum, sterility, identity and *in vitro* potency. All tests proved satisfactory.

During the preparation of these vaccine batches, certain additions and/or modifications of the previously employed manufacturing processes at CVL were introduced. The introduced changes led to improvement of both quantity and quality of the vaccine.

An *in situ* training programme of CBPP vaccine production and quality control personnel was formulated and implemented. This involved the demonstration of the various techniques used in the manufacture and testing of CBPP vaccine. The personnel were then allowed to repeat the demonstrated procedures under the supervision of the reporting officer. Additionally, write-ups of some of the protocols demostrated are included in this report for reference of the personnel.

Following detailed discussions wth CVL Staff on the recent post-vaccinal reactions associated with the use of CBPP vaccine in some parts of Mali, it seems quite probable that the vaccine used was based on the T1/44 seed strain which may have been administered through a non-recommended route to highly sensitive cattle breeds. The clinical picture described was consistent with post-vaccinal reactions associated with improper use of T1/44.

C. SUMMARY OF RECOMMENDATIONS

1. In view of the fact that the previously held seed stocks of CBPP vaccine were found not to be homogeneous in their content of viable mycoplasmas, these seed lots should be destroyed. CVL should then prepare future CBPP vaccines using the seed banks that were prepared during this consultancy mission. It is further recommended that due to the unpredictable and relatively adverse reactions of strain T1/44 (particularly if not properly administered) and the short duration of immunity engendered by the KH3J seed strain, CVL should manufacture only T1SR CBPP vaccine. The seed stocks should be stored at -20°C or lower and accurate records of usage should be maintained. The viability of the stocks should be checked after 2 years and if necessary a new seed lot can be prepared.

2. To facilitate easy monitoring of the pH of mycoplasma vaccine culture medium (a critical factor in mycoplasma viability), phenol red indicator should be incorporated in vaccine culture media. The consistently high titres that are expected should permit CVL to package CBPP vaccine as 50-dose vials, as is the case with rinderpest vaccine.

3. As local horse or donkey horse serum supports the growth of mycoplasma as well as similar imported sera, CVL may use the sera from its donkey or horse herds without compromising on vaccine quality or quantity. To ensure that the nutritive value of the serum is maintained, the serum should be correctly processed and stored. The use of locally obtained serum should result in considerable saving of mycoplasma media import costs.

4. To minimise wide batch to batch variations in the preparation of CBPP vaccine, mycoplasma culture harvests from different culture vessels should be pooled prior to repartition and lyophilisation. For this purpose a large (e.g. 30-40 litre capacity) glass vessel which is autoclavable should be acquired. An added advantage of pooling culture harvests is that batch sizes are increased and the cost of quality control testing decreased.

5. The current practice of diluting CBPP vaccine to a field dose prior to titration should be discontinued because such a procedure tends to result in underestimation of mycoplasma counts. Instead the procedure demonstrated during this consultancy mission should be used for all future mycoplasma vaccine titrations. Briefly, a vaccine vial is reconstituted in not more than 10ml of diluent, and subsequent dilution steps must involve change of pipettes or pipette tips between successive dilutions without touching the diluent meniscus with the tips or pipette.

6. Each vial of CBPP vaccine should be tested for vacuum before dispatch. The currently used system of water suction as a test for presence of vacuum cannot be applied for obvious reasons (there would no vaccine left). It is therefore necessary for CVL to use the available Edward's ST4M vacuum spark tester in assessing individual vaccine vials for presence of vacuum.

7. Although the vaccine dispensing/filling area is thoroughly washed and fumigated prior to use, the open environment in which this is done cannot be relied upon to always guarantee the sterility of the vaccine being handled. It is therefore strongly suggested that a large Class II laminar air flow cabinet be purchased so that vaccine can be filled under conditions which minimise chances of contaminations.

8. Autoclaving of 36% w/v suspension of skimmed milk stabilizer results in severe caramelisation, and this decreases the desired preservative effects of the stabilizer during lyophilisation. It is therefore recommended that skimmed milk for use in lyophilisation of CBPP vaccine be prepared as 20-25% w/v suspension and mixed with vaccine harvests so as to give a final skimmed milk concentration of 4-5% w/v.

9. To ensure that vaccine manufacture is not interrupted by machine breakdowns as witnessed during this period, it is advisable for the CVL to acquire an additional freeze dryer. The lyophilisation cycle used to freeze dry the current seed and vaccine lots may be adopted as it proved successful despite machine malfunction.

10. Glassware and other vessels used for mycoplasma culture should be thoroughly rinsed in distilled or purified water prior to sterilisation. Rinsing in tap water alone is not sufficient because certain water treatment chemicals in tap water e.g. chlorine are extremely mycoplasmacidal. For this reason CVL should have a reliable source of adequate supply of pure water. The present installed Millipore water purifier should be recomissioned for this purpose.

11. From time to time CVL should arrange for its CBPP vaccine production and quality control personnel to make training visits to reference/established vaccine manufacturing laboratories with a view to enabling them to keep abreast with technological developments in good laboratory and manufacturing practices which could then be adapted to suit local needs. In addition internal training sessions should be organised on a regular basis. These should permit reviews of the status of good laboratory (GLP) and manufacturing (GMP) practices in the laboratory.

12. The additional equipment indicated in the annex of this report should be purchased for use in the vaccine production and quality control laboratories. This will minimise possible chances of cross-contamination which can result from inter-laboratory loaning of equipment.

The CVL compares favorably with other African vaccine production laboratories (Ethiopia, Kenya, Botswana, Cameroon). However, if economically feasibly the CVL should consider broadening its range by making additional vaccines such as for poultry (e.g., New Castle disease), and it should aggressively market its products, both nationally and regionally.

D. INTRODUCTION

Contagious bovine pleuropneumonia (CBPP), is an economically important disease of cattle caused by *M. mycoides* subsp. *mycoides* (SC, bovine biotype). At one time or another in history it has occurred in Europe, Asia and America. Through a policy of strict restriction of animal (cattle) movement, slaughter and compensation, the disease has been eradicated from these continents. Australia stamped out the disease using a combination of vaccination, control of cattle movement and slaughter. CBPP remains endemic in Africa to this day.

Due to a variety of reasons (mainly geo-political, economic and/or socio-cultural), the methods that have been successfully used elsewhere appear not to be feasible in the African situation at this point in time.

It seems that vaccination is the most realistic option for the control of CBPP in Africa today. Currently CBPP vaccines used in Africa are freeze dried broth cultures of live attenuated *M. mycoides* subsp. *mycoides*. The seed strains used are T1/44 and KH3J or their streptomycin resistant variants namely T1SR and KH3J-SR respectively.

T1/44 (isolated in Tanzania from a mild case of CBPP in the 1950's) confers protection to immunised animals that lasts for at least one year. However, it still retains some residual virulence to some breeds of cattle particularly if it is not administered through "safe" routes.

T1SR (developed by subjecting T1/44 to three successive passages in media containing increasing concentration of streptomycin), is claimed to be comparable to T1/44 in its immunogenic potency and yet causes less severe post-vaccinal reactions.

The most attenuated strain (KH3J), was first isolated in southern Sudan. Both KH3J and KH3J-SR are known to cause no vaccination reactions to all breeds of cattle, irrespective of the route or number of mycoplasmas used. The two strains however, give protective immunity that lasts only for about 6 months, and this necessitates biannual vaccinations. Because of the short duration of immunity of the KH3J and KH3J-SR, these seed strains have largely been abandoned in the production of CBPP vaccines in Africa today.

To be effective in the control of CBPP, a vaccination exercise must ensure the use of good quality vaccines, high immunisation coverage (at least 80%) and regularly repeated vaccinations over long periods of time.

The draft OIE norms for CBPP vaccine stipulate that the vaccine should:

a) Be produced on a seed lot system. The seed used must be shown to be free from bacterial, fungal and other adventitious agents contamination. The identity of *M. mycoides* subsp.

mycoides must be confirmed.

b) Be demonstrated to contain at least 10 million viable mycoplasmas per cattle (field) dose.
c) Be proved to be innocuous to laboratory animals and safe for use in cattle.
d) Give protection to immunised cattle for at least one year.

The Central Veterinary Laboratory (CVL) and the Animal Productivity and Export project (APEX) in Mali are working in collaboration with the Washington State University (WSU) Collaborator Group to improve the performance of the livestock sector. A key element of these activities is the production of good quality vaccines, including the vaccine for the prevention of CBPP. The recent re-emergence of CBPP in Mali and in many other parts of Africa has resulted in the decision by CVL and APEX to consider the control of CBPP as a high priority activity.

However, in the recent past, CVL has been experiencing problems in the production of CBPP vaccine, particularly in trying to consistently achieve required potency of the vaccine. Problems have also been experienced at the field level in that severe post-vaccinal reactions occurred following the use of at least one batch of CBPP vaccine prepared at the CVL.

This report presents the summary of works undertaken and results obtained during a consultancy mission to the CVL, Bamako, Mali. The primary objective of the mission was to assist the CVL in its efforts aimed at producing good quality CBPP vaccine.

E. SPECIFIC OBJECTIVES

In collaboration with the personnel in the Vaccine Production Division, the consultant will:

1. Set up and establish control procedures for a "banque de semence" (vaccine reference bank).

2. Prepare some lots of bovine mycoplasma vaccine with the lines KH3J and T1.

3. Ascertain and assure quality control of lots produced.

4. Assist the CVL to gain a better understanding of the causes of the recent post-vaccinal reactions and how resolution and prevention of future occurences can best be achieved.

5. Define and conduct on-the-job (action training) for technical personnel in the unit.

F. ITINERARY

DATE	DEPART	ARRIVE	FLIGHT No.
22/07/95	Nairobi (08.15)	Addis Ababa (10.15)	ET 950
22/07/95	Addis Ababa (11.55)	Bamako (18.00)	ET 963
10/09/95	Bamako (21.55)	Addis Ababa (09.40)	ET 972
12/09/95	Addis Ababa (10.15)	Nairobi (12.15)	KQ 403

N.B. : The original contract of this consultancy mission was to have ended on 22 August 1995. However, during a debriefing meeting held on 17th August 1995 and attended by officers from CVL, APEX project, USAID and the consultant, the CVL recommended that this contract be extended by an additional two weeks to enable the consultant to produce two lots of CBPP Vaccine and to supervise the quality control testing of the lots prepared.

G. WORK DONE AND RESULTS

1. SPECIFIC OBJECTIVE 1: ESTABLISHMENT AND TESTING OF "BANQUE DE SEMENCE"

The OIE recommends that the production of CBPP vaccine should be based on a seed lot system. This is a system according to which successive batches of product (i.e. CBPP vaccine) are prepared using the same master seed lot.

The Central Veterinary Laboratory (CVL) has been producing CBPP vaccines based on T1SR, KH3J and T1/44 seed lots. Its main complaint was the decrease in titre of its CBPP vaccine seed banks (established 3 years ago). Also, in the recent past (1994) severe post-vaccinal reactions and cattle mortalities followed administration of batch 99/94 of CBPP vaccine which had been prepared at the CVL.

The establishment of fresh and tested seed lots was therefore deemed necessary to avoid these problems. Three seed lots based on T1SR, KH3J and T1/44 were therefore prepared.

(i) Materials and Methods

Mycoplasma master seeds and origins :
T1SR : At passage level 49 was obtained from EMVT, France.
KH3J : Obtained from EMVT at passage level 87.
T1/44: Obtained from Kenya Vaccines Institute, Muguga, (passage level 45 and lyophilised in 1959)

Mycoplasma Seed/Culture Medium: Newing's tryptose broth medium (popularly known as Gourlay bacto-tryptose broth medium) was used for the seed mycoplasma cultivation as well as for the preparation of vaccine lots. The ingredients for 1 litre of this medium are :

Donor Horse serum (Sigma)	100 ml
Bacto tryptose	20 g
Dextrose	2 g
Sodium chloride	5 g
Disodium hydrogen phosphate (anhydrous)	2.5 g
Yeast extract	1 g
Glycerol	5 ml
Penicillin	2 million units
Phenol red	0.02 g
Distilled water to:	1000 ml

N.B.: Medium pH was adjusted to 7.8 using 1M NaOH. In addition the medium destined for the cultivation of T1SR contained streptomycin at a final concentration of 0.2 mg/ml. Medium ingredients were dissolved by magnetic stirring and then sterilised by passing it through a Seitz filter. Filtered medium was tested for sterility and kept at +4°C until use.

Mycoplasma agar medium: The ingredients for 100ml of medium are:

Mycoplasma agar base	2.55 g
Dextrose	0.10 g
Agar	1.0 g

Yeast extract	0.1 g
Distilled water	85 ml

These were mixed by magnetic stirring and pH adjusted to 7.8 using 1M NaOH. Medium was then autoclaved at 120°C for 15 minutes and placed in a water bath set at 55°C. A separate container with horse or donkey serum (10 ml), penicillin (20000 IU), and 0.2% DNA solution (1ml) was placed in the water bath at 55°C. All media ingredients were then allowed to equilibrate in the water bath. In a laminar air flow cabinet horse serum, DNA and penicillin mixture were addded to the molten agar base medium.

The homogeneous mixture was then dispensed into sterile 90x15mm Petri dishes at 10 ml/dish and the agar was allowed to set. The agar plates were placed in a can with lid or wrapped in aluminium foil and stored at +4°C until needed.

Agar gel for immunodiffusion test:

Agar	1.2 g
0.01M phosphate buffered saline	100 ml (pH 7.4)

The agar was dissolved in the stated volume of saline by autoclavation (120°C for 15 minutes). 10ml of the molten agar was poured into each 90x15mm glass Petri dishes and agar allowed to set on a horizontal surface in a laminar air flow cabinet. Plates containing the solidified agar gels were then stored in a refrigerator until required.

Media for sterility testing : Fluid thioglycollate and Soya bean Casein digest (trypticase soya broth) media were reconstituted as described by the manufacturer.

Each medium was then dispensed into screw-capped glass tubes (5 ml/tube) and sterilised by autoclavation (120°C/15 minutes). Autoclaved media were incubated at 37°C for 24 hours and then stored in a refrigerator until use.

Medium for testing streptomycin resistance: This was prepared as described for mycoplasma broth culture medium except that streptomycin was added to a final concentration of 1 mg/ml prior to immediate use.

Skimmed milk (freeze drying stabilizer): Skimmed milk is the most commonly used freeze drying stabilizer in CBPP vaccines in Africa. This is because skimmed milk is cheap, readily available, effective and dries fast. For use in CBPP vaccine lyophilisation, the CVL prepares skimmed milk as a 36% w/v concentration which is then sterilized by autoclaving and mixed with vaccine culture to give a final concentration of the stablizer of 3%.

During this period a 36% concentration of skimmed milk sterilized by autoclaving (120°C/15 minutes) was found to be severely caramelised. Caramelised milk coupled with the low concentration (3%) used by the CVL could have contributed to reported high loss of CBPP vaccine titres during lyophilisation. This might have arisen because the stabilising effect of skimmed milk was not optimum.

To determine the highest concentration of skimmed milk that could be prepared without severe caramelisation, 10%, 20% and 25% w/v suspensions were sterilized by autoclaving and then visually assessed for consistency and colour changes as evidence of caramelisation. The 10% and 20% suspensions retained their colour. Although there was slight evidence of caramelisation of the 25%

suspension this was thought not to be serious enough as to adversely affect the stabilising effect of thee milk. For use in freeze drying the seed lots and the vaccine batches during this mission 20 or 25% skimmed milk suspensions were prepared and mixed with mycoplasma culture harvests so as to obtain a final skimmed milk concentration of 4%.

It is recommended that CVL adopts this scheme of preparation and use of skimmed milk for its future lyophilisations of CBPP vaccine. Prepared skimmed milk must be tested for sterility and kept refrigerated until use.

Laboratory animals: Mice and guinea pigs were obtained from the colonies maintained at the CVL.

Hyperimmune serum: *M. mycoides* subsp. *mycoides* (strain PG1) reference hyperimmune serum from EMVT.

(ii). Mycoplasma Seed Culture Procedures

Reconstitution: An ampoule containing each of the freeze dried mycoplasma master seed was thoroughly disinfected externally with 70% ethanol and the top third cut out aseptically using a glass cutter. Sterile distilled water was then added to reconstitute the lyohilised seed (5ml for T1SR, 3ml for KH3J and 3ml for T1/44).

Inoculum preparation: Using 1ml of reconstituted seed ten-fold dilution series were prepared in tubes contanining 9ml of mycoplasma broth medium (10^{-1} to 10^{-7}). 1ml of each dilution was then subinoculated into a series of 5 tubes each containing 9ml of medium. Inoculated tubes were incubated at 37°C for upto 8 days. Begining from day 5 following incubation tubes showing evidence of mycoplasma growth (i.e. change of medium colour from pink to yellow) were removed from the incubator, appropriately labelled and kept at +4 °C.

Tubes of each seed strain culture were then pooled and each pool constitued the inoculum. Reconstituted seed and the inoculum were tested for sterility.

CVL Seed bank culture: 70 ml of each seed strain inoculum was used to seed 630ml of growth medium in 1 Litre bottles. The inoculated media bottles were then incubated at 37°C. 24 hours post-incubation each culture was agitated by magnetic stirring (70 rpm) until harvested. The harvest times post-incubation and passage levels of the cultures were:

T1 SR - 44 hours (Passage 51)
KH3J - 64 hours (Passage 89)
T1/44 - 69 hours (Passage 47)

Lyophilisation: To each seed strain culture, 20% sterile skimmed milk was added so as to give a final stabilizer concentration of 4% (e.g.120ml of the milk would be added to 480ml of culture harvest). Antigen-stabilizer mixture was homogenised by magnetic stirring and then dispensed into 17ml freeze drying vials with each vial receiving 2.5ml of seed culture. The seed was then freeze dried in a Virtis freeze dryer model 251 SRC7 following the protocol described in Annex I of this report. Lyophilised seed culture vials were appropriately labelled and stored at -20°C under lock and key.

N.B. After dispensing samples from each seed type vials were taken for mycoplama titration and sterility testing.

(iii). Quality Control Tests on the "Banque De Semence"

Each seed type of the freeze dried mycoplasma seed bank was subjected to the following tests:

Estimation of viable mycoplasma content: The number of viable mycoplasma counts was estimated by determination of the median effective dose in a multiple serial dilution assay. This was carried out on U-shaped or flat-bottomed 96 well microplates.

Briefly the lyophilised contents of a vial of the seed were reconstituted in 5ml sterile and cold distilled water. Serial decimal dilutions of the reconstituted vaccine (10^{-1} to 10^{10}) were then prepared in tubes of base mycoplasma broth medium (i.e. mycoplasma broth medium without serum or antibiotics) as diluent.

From each dilution 100microlitres or 0.1ml was added into each of ten microplate wells. Each well of the microplate contained pre-added 100microlitres of mycoplasma broth medium. A set of 8 uninoculated medium wells were included as negative controls. Inoculated plates were sealed and incubated at 37°C for 10 days. The plate was then examined for evidence of mycoplasma growth as indicated by change of medium colour from pink to yellow. The number of viable mycoplasma counts was calculated using the Spearman-Karber formula and expressed as Log_{10} colour changing units $_{50}$ (Log_{10} CCU_{50}).

The test for sterility: The reconstituted master seed, pooled inoculum, seed harvest-skimmed milk mixture and the lyophilised "banque de semence" were each tested for sterility.

The test, designed to detect presence of bacterial and/or fungal contamination involved the inoculation of aliquots of the sustance under test into tubes of thioglycollate and tryptic soya broth media. A portion of the inoculated soya broth tubes was incubated at room temperature while the other portion together with the thioglycollate medium were incubated at 37°C. Incubation lasted for 14 days when final visual examination of the media was made. Presence of contamination would be indicated by turbidity of the sterility test media.

N.B. As skimmed milk used for lyophilisation clouds the sterility test media, the sterility media inoculated with freeze dried seed and liquid seed-skimmed milk mixtures were incubated for 7 days and then subinoculated into fresh tubes of corresponding media. The latter were then incubated for a further 7 days before taking the final reading. For each test appropriate diluent and media controls were included. The test was considered to be valid if none of the controls showed evidence of contamination. If any control medium tube shows contamination the test is invalid and must be repeated.

Test for vacuum: The presence of vacuum in a freeze dried vaccine vial was assessed by immersing the vial in a bucket of water and then puncturing the vial rubber stopper with a needle. Vacuum is indicated by filling of the vial with water through suction. Later on it was actually found that the CVL has an Edward's ST4M vacuum spark tester which should used for all future vacuum assessments.

The test for streptomycin resistance: A vial of each seed strain was reconstituted using 5ml of cold and sterile distilled water. 0.5ml of the diluted vaccine was inoculated into each of 2 tubes containing 5ml of mycoplasma broth. The test medium contained streptomycin at a concentration of 1mg/ml. Streptomycin resistant strains of *M. mycoides* should grow in such a medium and such growth is indicated by change of medium colour from pink to yellow. In performing this test

appropriate diluent and uninoculated medium controls were set up, and the test was considered valid if none of the medium and diluent controls changed colour.

Agar gel immunodiffusion test: The test was performed by letting hyperimmune CBPP reference antiserum and the mycoplasma seed antigen to diffuse towards each other from wells in agar gels. Briefly O.02ml of antiserum was added into a central well while O.O2ml of mycoplasma antigen (seed reconstituted in 5ml of distilled water) was added into the peripheral wells. Distances between wells were about 3.5mm and the gel punch used had an internal diameter of about 4mm.

The reactants were allowed to diffuse against each other for 48 hours in a moist chamber at room temperature. A negative control (broth medium) and a positive control (known *M. mycoides* subsp. *mycoides* culture) were included in each test. The appearance of a line of precipitation between the antigen and antiserum wells was considered as a posititve identification of *M. mycoides* the seed strain species.

The test for mycoplasma growth inhibition: This test is derived from the observation that growth of mycoplasma colonies on agar is inhibited by high titre homologous hyperimmune serum. It is used for the specific identification of mycoplasma species.

Each seed was diluted in sterile distilled water to contain about 10^4 to 10^5 mycoplasmas per millilitre. 0.2ml of the diluted sample was placed at one end of a 90x15 mm mycoplasma agar plate. By gentle tilting of the plate, the mycoplasma containing fluid was allowed to spread uniformly over the entire agar surface.

Using a gel punch (4mm intemal diameter) a well was cut in the agar. 0.03ml of CBPP hyperimmune serum was added into the well and the plate incubated at 37 °C in a moist chamber for 10 days.

Agar plates were then examined under an inverted microscope. The presence and diameter of mycoplasma colony growth inhibition around the antiserum were recorded. Observation of growth inhibition indicates a specific identification of *M. mycoides* subsp. *mycoides*.

The test for innocuity: A vial of the freeze dried seed was reconstituted in 2.5ml of sterile and cold distilled water. Groups of guinea pigs and mice were then inoculated as shown in the following table:

Table I: Inoculation of Laboratory Animals

Inoculum	Guinea pigs (ip)	Mice (ip)	Mice (sc)
T1SR	2	3	3
KH3J	2	3	3
T1/44	0	3	3
Controls	2	3	3

(ip) = inoculation via intraperitoneal route
(sc) = inoculation via subcutaneous route

The volumes of the seed vaccine used were 0.5ml for guinea pigs and 0.25ml for mice.

The test animals were kept under observation for 30 days. At the end of this period the animals are to be sacrificed and complete post mortems carried out.

(iv). Results of Tests on the Seed Lots

Mycoplasma titres: The content of viable mycoplasmas of the seed lots were as shown in **Table II** below.

Table II: Titres of Seed Lots

Seed strain	Titre/Vial (Log_{10} CCU_{50})
T1SR	10.7
KH3J	10.6
T1/44	8.6

Sterility: The observations were as summarised in Table III below.

Table III: Sterility Test Results

Seed strain	Incubation at room temperature	Incubation at 37°C
T1SR, T1/44 and KH3J Master seeds	NEC	NEC
T1SR	NEC	NEC
KH3J	NEC	NEC
T1/44	NEC	NEC

NEC = No evidence of bacterial or fungal contamination

Streptomycin resistance test: The observations were as shown in **Table IV**.

Table IV: Streptomycin Resistance Test

Seed strain	Test result
T1SR	+ve
KH3J	-ve
T1/44	-ve

+ve = resistant to streptomycin
-ve = not resistant to streptomycin

Agar gel immunodiffusion: Results obtained were as shown in **Table V**.

Table V: Immunodiffusion Results

Seed strain	Test result
T1SR	+ve
KH3J	+ve
T1/44	+ve

+ve = Appearance of a line of precipitation between antiserum and antigen wells within 48 hours of incubation of agar gel plates

The test for vacuum: All the vials punctured under water got completely filled with water. This indicated presence of good vacuum.

Mycoplasma growth inhibition test: Inoculated mycoplasma agar plates were inadvertently removed from the incubator and placed in a refrigerator. As a result any mycoplasma growth that may have occurred was washed off the agar surface by condensation fluid that had accumulated. This test has been repeated but this time with a drop of the hyperimmune serum placed on the surface of the mycoplasma-inoculated and dried agar. Reconstituted vaccine seed was not diluted prior to inoculation of agar plates.The results of the repeat test were as shown in **Table VI** below.

Table VI: Growth Inhibition Test Results

Seed strain type	Result
T1SR	+ve
KH3J	-ve
T1/44	+ve

+ve = presence of mycoplasma colony growth inhibition
-ve = No observable zone of growth inhibition

Growth inhibition was taken as complete absence of growth or a decrease in the number and size of mycoplasma colonies around the antiserum drop. The KH3J seed strain is known to possess many colonies breaking through inhibition zones and this coupled with the high seeding rate used in the present test could have resulted in the negative result above. It is suggested that the test in respect of KH3J be repeated using a 10^{-4} dilution of the contents of one vial of the seed stock

The test for innocuity in laboratory animals: Up to the time of the preparation of this report all inoculated and control mice remained healthy. One guinea pig that had been inoculated with T1SR seed strain died 3 days after inoculation. At post mortem the carcass was found to be partly decomposed. Nevertheless spleen, kidney and liver samples were taken for bacteriological or mycoplasma culture. This was to establish whether the cause of death was related to the inoculated mycoplasma seed. The samples taken were negative for mycoplasma isolation.

The other bacteriological tests are still on-going. As there were no more guinea pigs available to facilitate a repeat test, it is suggested that CVL take steps to perform a further guinea pig inoculation test of the seed stocks and the vaccine lots prepared.

Digitonin sensitivity test: It would have been desirable to carry out this test on the seed stocks as a screen for any Acholeplasma contamination. However, due to unavailability of digitonin at the time of this consultancy the test could not be performed. It is suggested that CVL arrange to do this test as soon as it has acquired digitonin

DISCUSSION

The detailed descriptions of the quality control procedures on the prepared CBPP vaccine seed lots and subsequent vaccine batches are contained in the "Pan African Veterinary Vaccine Centre (PANVAC) Standard Operating Procedures for the quality control testing of CBPP Vaccine", a copy of which was availed to the CVL. It is recommended that the described methods be adopted by the CVL for all its future in-house quality control testing of CBPP vaccines.

A good starting point is the destruction of all seed stocks previously maintained by CVL and using only the prepared banks for future vaccine preparations. During discussions with staff it was indicated that CVL (for the time being) wishes to produce only T1SR-based CBPP due to vaccine potency and safety considerations as well as endeavouring to satisfy consumer preferences of the regions served by this laboratory. This proposal is sound. However, it should be borne in mind that although T1SR is known to be safe for use in cattle (causing only transitory reaction at inoculation site), it is nevertheless still advisable to confirm this safety at least at the laboratory level.

2. SPECIFIC OBJECTIVE 2: PREPARATION OF SOME LOTS OF CBPP VACCINE

(i). Background

During discussions with vaccine production and quality testing staff the following complaints pertaining to CBPP vaccine were raised:

- Falling titre of the CBPP vaccine seed which was prepared in 1992.
- Wide variations of vaccine culture titres from one batch to another and from one culture vessel to another.
- Low mycoplasma titres of liquid vaccine harvest.
- High titre losses (more than 1 log.) of vaccine during lyophilisation (4-5 day cycle).

An examination of CVL procedures for the preparation of CBPP vaccine revealed the following as possible causes of the problems:

- Samples from the seed lot previously used were titrated and tested for sterility. The seed was found to be sterile, but while some vials had titres of $\log_{10}$ 9.5 CCU_{50} some had very low titres (less than $\log_{10}$ 3.5 CCU_{50}). This would suggest that the seed lot was not uniform in so far as viable mycoplasma content goes. Use of such a seed lot can lead to considerable vaccine batch variations as experienced by the CVL.
- Glassware used for CBPP vaccine preparation were washed and rinsed using tap water only. It is known that certain water treatment chemicals that may be contained in tap water e.g. chlorine, can be extremely mycoplasmacidal. This may explain the variation in vessel to vessel mycoplasma yields.
- Donor horse or donkey serum (for incorporation into mycoplasma growth media) was often kept unharvested for too long at +4°C. This decreases the nutritive value of the serum and easily leads to erythrocyte lysis which will then discolour media, thus making visual monitoring of vaccine culture pH quite difficult.

- Reconstituted mycoplasma seed was directly inoculated into bulk vaccine culture media. The culture would then be incubated (at 37°C) stationary for 24 hours when it was agitated by magnetic stirring (130 rpm) for a further 48 hours when it was harvested and freeze dried. pH monitoring was not done. It is likely that under these culture conditions the culture pH at the time of harvest would have dropped to below 6.7; the lowest value that assures good mycoplasma vaccine titre. Culture media are best inoculated with seed mycoplasma at logarithmic phase of growth. This decreases lag phase during growth of mycoplasmas and leads to higher antigen yields.
- Skimmed milk stabilizer was being prepared as a 36% suspension and used for freeze drying at a final concentration of 3%. This (36%) concentration of skimmed milk easily caramelises on autoclaving and therefore cannot give optimum stabilising effect.
- Freeze drying cycles used to lyophilise CBPP vaccine varied from one batch to another.This could also have contributed to further variations and the high losses (up to 10%) of vaccine during some freeze drying runs.

These observations were therefore taken into account during the preparation of the following lots of CBPP vaccine.

(ii). Materials and Methods

Medium: Bacto-tryptose broth with 15% horse or donkey serum. Medium ingredients were prepared as described for the seed bank. The medium was filtered into sterile 10L flat-bottomed glass baloon flasks with each flask receiving 5L of medium.Each flask was sampled for sterility testing and the baloons kept at +4°C to await test results.

NB: The incubation of media at 37°C immediately after filtration as previously done at CVL does not offer the chance of resterilisation should the medium be found to be contaminated.

Preinoculum: This was a pool of T1-SR seed culture grown in tubes containing 9ml medium and harvested at exponential phase of mycoplasma growth (usually after 3-5 days).

Inoculum: To prepare the inoculum, growth medium was inoculated with a preinoculum quantity equal to 1/10 the volume of medium. This was then incubated at 37°C for 24 hours, agitated by magnetic stirring for a further 20 hours and then harvested for use in preparing bulk vaccine cultures.

Bulk cultures: Each baloon flask containing 5L of medium was seeded with 500ml of inoculum. Flasks were then incubated at 37°C. After 24 hours of stationary incubation culture agitation was commenced (100rpm) and incubation continued for an additional 46 hours (for batches CM3, CM4 and CM7) or 20 hours (for batchesA/D, E/B and C/F) when harvests were made. Culture pH was measured at the harvest time. Just before lyophilisation, skimmed milk was added to the harvests to a final 4% concentration.

Batches CM3, CM4 and CM7 represented the harvests of 3 baloon flasks. They were dispensed into 17ml vials (at 4ml/vial). Each of batches A/D, E/B and C/F was a pool of two flasks. These were dispensed into 17ml vials with each vial receiving 3ml.

Lyophilisation procedure was as described for seed lots except that during these runs freeze dryer shelf heating was applied. At the end of each freeze drying run, vaccine vials were sealed with aluminum caps, samples for quality control taken and the rest of the vials stored at -20°C.

DISCUSSION

The key thing in CBPP vaccine preparation is that the glassware used for the cultivation of mycoplasma should be thoroughly rinsed in distilled water in order to remove any traces of substances that would inhibit the growth of mycoplasma.

The media ingredients should be of the right quality and correctly processed. The water used to reconstitute these ingredients should be of high purity.

Serum is an indispensable additive in mycoplasma media as it supplies cnolesterol which is essential for the maintenance of mycoplasma membrane integrity. It also supplies other mycoplasma growth requirements. Before use, serum must be shown to be free of mycoplasma growth inhibitors, and it should be correctly processed. Horse or donkey serum have been shown to support mycoplasma growth quite well and either can be used with satisfactory results. Serum harvests are best dispensed in small volumes (e.g. 500ml quantities) and then stored frozen (-20°C) until use. This will permit thawing and immediate use of only the required quantity of serum. Frequent thawing and refreezing of serum decreases its quality and should thus be avoided.

During the preparation of the vaccine batches above, a mixture of horse and donkey serum that may have been thawed too many times and/or kept at +4°C for too long was used to supplement the mycoplasma media. Consequently, this may have resulted in the comparatively lower titres of the vaccine batches relative to those of the seed lots. In the preparation of the seed lots, horse serum supplied by Sigma Chemicals Company was thawed and used immediately. The validity of the above suspicion will be proved by the results of the next batch of vaccine in which serum processed as suggested was used.

The preparation of a common liquid inoculum for seeding bulk media flasks was used to assess flask to flask variation in antigen yields. The titre results obtained from different flasks seem to indicate that provided the same ratio of inoculum to medium volumes were used there was no significant variation from one flask to another.

The pooling of culture harvests was aimed at increasing a batch size, minimising batch to batch variations and the reduction of the cost of the quality control tests.

3. SPECIFIC OBJECTIVE 3: TO ASCERTAIN AND ASSURE QUALITY CONTROL OF LOTS PRODUCED

Each batch/lot of the prepared vaccine was subjected to the following quality control tests:

(i). Sterility: after addition of stabilizer and after freeze drying
(ii). Vacuum: on freeze dried vaccine
(iii). Titration: before and after lyophilisation
(iv). Agar Gel Immunodiffusion: on freeze dried vaccine
(v). Streptomycin Resistance: on freeze dried vaccine
(vi). Growth Inhibition: The materials and test procedures were as described for the quality control testing of the "banque de semence".

RESULTS

(i) Vacuum: The observations are shown in **Table VII.**

Table VII: Vacuum Test Results

Vaccine lot	Result
CM 3	Pass
CM 4	Pass
CM 7	Pass
A/D	Pass
E/B	Pass
C/F	Pass

Pass = Presence of vacuum

(ii). Sterility: The results were as indicated in Table VIII. below.

Table VIII: Sterility Test Results

Vaccine Lot	Before freeze drying	After freeze drying
CM3	Pass	Pass
CM4	Pass	Pass
CM7	Pass	** Fail
A/D	UT	UT
C/F	UT	UT
E/B	UT	UT

UT = sterility test still going on

** Fail = first test indicated contamination and the test for sterility is to be repeated

(iii). Mycoplasma Titration Results: These were as indicated in **Table IX**.

Table IX: Titration Results (expressed as Log_{10} CCU_{50})

Vaccine lot	pH at harvest	Before freeze dying (per ml)	After freeze drying (per ml)	After freeze drying (per vial)	Per Dose for 50-dose vials
CM3	6.66	9.30	9.19	9.80	8.10
CM4	6.62	9.30	8.64	9.35	7.65
CM7	6.62	9.60	8.79	9.50	7.80
A/D	A=6.83 D=6.84	9.45	9.03	9.50	7.80
C/F	C=6.87 F=6.52	9.60	9.13	9.60	7.90
E/B	E=6.81 B=6.80	9.70	8.93	9.40	7.70

(iv). Agar Gel Immunodiffusion: Results are given in **Table X**.

Table X: Immunodiffusion Results

Vaccine lot	Result
CM3	+ve
CM4	+ve
CM7	+ve
A/D	UT
C/F	UT
E/B	UT

+ve = appearance of precipitin line between antigen and antiserum wells
UT = under test

(v). Streptomycin Resistance Test

Table XI: Streptomycin Resistance Test Results

Vaccine lot	result
CM3	Pass
CM4	Pass
CM7	Pass
A/D	Pass
C/F	Pass
E/B	Pass

Pass = growth in medium containing 1mg/ml of streptomycin

(vi). Growth Inhibition Test

Table XII: Growth Inhibition Results

Vaccine lot	result
CM3	Pass
CM4	Pass
CM5	Pass
A/D	Pass
C/F	Pass
E/B	Pass

Pass = presence of a zone of mycoplasma colony growth inhibition around the antiserum drop.

(vii). Observations During and After Lyophilisation

First lyophilisation run: This was used to freeze dry the prepared seed lots. No shelf heating was applied throughout the lyophilisation process. Only one shelf was used. The number of seed vaccine freeze dried were: 322 (T1SR), 304 (KH3J) and 309 (T1/44). There was good cake formation, the cake easily detached from vial bottom and was easily crumbled when crushed in a piede of paper (these were subjective means of assessing the efficacy of the lyophilisation process).

Second lyophilisation run: Prior to this run one of the compressors of the freeze dryer which had broken down was replaced. Subsequently lyophilisation was carried out with shelf heating. The final vacuum reading was not as good as during the previous run and one of the condensers was not cooling as well as during the previous run. 4079 vials of T1SR vaccine were prepared. The drying was not very good and this vaccine should not be stored for too long i.e. it should be used within 6 months because the high residual moisture cannot permit prolonged storage. 1% of the vials were spoiled mainly through breakages due to structural weakness of the vials used.

Third lyophilisation run: A total of 10596 vials of T1SR vaccine were freeze dried during this run. Shelf heating was applied. The compressor that had been replaced previously developed problems in the middle of the lyophilisation run and had to be disconnected and the freeze drying proceeded to completion with one compressor. There was a difference of 4°C between the two condenser temperatures. Despite these malfunctions there was good freeze dried cake relative to the second lyophilisation run. **The cause of the frequent compressor breakdown should therefore be investigated and rectified**. The breakdown also reiterates the need to have a back-up freeze dryer.

4. SPECIFIC OBJECTIVE 4: TO ASSIST THE CVL TO GAIN A BETTER UNDERSTANDING OF THE CAUSES OF THE RECENT POST-VACCINAL REACTIONS AND HOW RESOLUTION AND PREVENTION OF THE FUTURE OCCURENCES CAN BEST BE ACHIEVED

(i) Background

Severe post vaccinal reactions and cattle mortalities occurred following the inoculation of batch 99/94 produced at the CVL in July 1994 into cattle in two regions of Mali. The cattle breeds affected were N'Dama, Zebu and N'Dama-Zebu crosses. Perusal of production, quality control records and veterinary investigation reports by CVL personnel and central government officials showed the following:

Production records:

Batch number:	99/94
Seed strain:	*M.mycoides* (T1SR strain)
Number of baloons:	4
Number of doses:	335,120
Growth medium:	Gourlay with horse serum
Doses per vial:	40
Freeze drying stabilizer:	Skimmed milk (3%)

Quality control results done at CVL before Vaccine dispatch:

Sterility:	passed
Innocuity:	passed
Vacuum:	passed
Titre per dose:	$10^{7.21}$ mycoplasmas

Safety tests at CVL after reports of post-vaccinal reactions:

Innocuity in laboratory animals:	passed
Safety test in cattle:	passed

Results of PANVAC quality control tests (samples submitted after vaccination reactions):

Innocuity in mice:	passed
Innocuity in guinea pigs:	passed
Titre:	failed
Sterility:	passed

Findings of investigations into the post-vaccinal reactions:

Areas affected: Sikasso and Kayes

Kayes area: 1716 cattle were vaccinated out of which 82 animals died. Also within this region 480 cattle in one village were vaccinated and none of them showed any post-vaccinal reactions.

Sikasso area: out of 1124 cattle vaccinated in one village 139 animals died. Within this area a total

of 50,000 cattle had been vaccinated and it is only in the village above that mortalities occurred.

Samples submitted from these areas were tested for precipitating CBPP antigen and found to be positive. Histologically the major lesions were exudative bronchopneumonia, haemorrhages and multifocal necrosis. On this basis CBPP was diagnosed.

With the above facts in mind several explanations emerge as possible causes of the observed post vaccination reactions. These include:

(i). **Vaccination of animals that were incubating CBPP:** The presence of lesions normally found in CBPP cases in the samples submitted from the areas affected lends credence to this possibility. The positive agar gel precipitation reaction however, cannot provide conclusive evidence of CBPP because this test cannot distinguish between vaccinal strain antigen and that of wild/virulent *M. mycoides* subsp. *mycoides.* The isolation and identification of mycoplasma from the suspect lung samples should have been attempted in this particular case.

(ii). **Use of vaccine contaminated with wild/virulent strains of CBPP agents:** This is unlikely to have occurred because innocuity tests in laboratory animals at PANVAC and at CVL did not support this possibility. Besides, at CVL vaccine production units are completely separated from other units such as the diagnostic laboratories. Personnel in the vaccine unit do not work elsewhere and personnel from other units are not allowed into the vaccine production units. Neither are potentially infective materials such as field samples for diagnosis handled in the vaccine production premises.

(iii). **The vaccine used in areas that experienced reactions may have been based on T1/44 and administered through non-recommended route:** This is thought to be one of the likely explanations of what may have happened and the following reasons would seem to support this thinking:

- It is known that T1/44 still retains some residual virulence and it is for this reason that the recommended route of inoculation of T1/44 is subcutaneously at the tail tip. Even then there are still some localised reactions and 1-2% of vaccinated cattle may lose tails.
- Certain breeds of cattle e.g. the N'Dama, are extremely sensitive to T1/44. In the areas that experienced reactions, N'Dama, Zebu, and Zebu-N'Dama cross breeds had been vaccinated.
- CVL has been producing both T1SR and T1/44 based CBPP vaccines. The present head of CBPP vaccine unit and the chief of vaccines unit were out of CVL when batch 99/94 was prepared. Consequently they cannot unequivocably rule out the possibility of a mix-up of T1SR and T1/44 during vaccine culture production or during labelling of freeze dried vaccine.
- The localised Willems-type lesions without accompanying lesions in the lungs and the short duration of the onset of the reactions can only suggest that the cause of the lesions must have been caused by something contained in the substance that was inoculated.

Attempts to retest samples of batch 99/94 during this consultancy were not possible because the remaining vaccine had been sealed by central government authorities pending arrangements to destroy it.

RECOMMENDATIONS

Based on the above assumptions the following would ensure future non-recurrence of post-vaccinal reactions:

- Use of only T1SR or KH3J to prepare CBPP vaccine. Should T1/44 be used it should be administered at the tail tip, and users and stock owners must be made aware of the possibility of occurence of post vaccinal reactions.
- The prepared seed stocks should be kept under lock and key. Accurate records of usage of each vial of seed stock utilised for whatever purpose should be strictly maintained.
- As a routine practice, all batches of CBPP vaccine should be tested for safety in cattle before dispatch.
- The mechanisms for recall of vaccines from the field in the event that a vaccine is found to be unsuitable should be strengthened.
- Education of field veterinarians and paraveterinarians on the proper handling and use of vaccines should be pursued vigorously. The CVL should take a lead in this activity.
- The CVL should always closely monitor field vaccination campaigns to ensure timely intervention should this be necessary.

5. SPECIFIC OBJECTIVE 5: DEFINE AND CONDUCT ON-THE-JOB (ACTION-TRAINING) FOR TECHNICAL PERSONNEL IN THE PRODUCTION UNIT

Initial discussions with staff were used to assess the level of understanding of personnel in the field of CBPP vaccine production and quality control. In the opinion of the consultant they were found to have fair knowledge of the field although certain weakness in technique application were noted.

The following strategy was therefore adopted in conducting *in situ* training of staff:

a) Theory and principles of procedures behind CBPP vaccine production and testing.
b) Demonstration of the technique by the consultant in presence of staff.
c) Performance of demonstrated technique by staff under the supervision of the consultant. This permitted on-the-spot advice on any observed shortcomings.
d) Discussion of the results obtained and interpretation of same.
e) The need to keep records and the type of records to be kept during vaccine production and testing of CBPP vaccine.

Using this model as a base for the action-training the following procedures were covered:

- preparation of glassware and other equipment
- mycoplasma media preparation and testing
- preparation of seed inocula and testing
- bulk vaccine culture preparation
- preparation, sterilisation and testing of skimmed milk stabilizer
- lyophilisation of vaccines with special emphasis on CBPP vaccine
- quality procedures of lyophilised vaccines (titration, sterility, vacuum, identity, safety and innocuity)
- interpretation of test results
- design and use of records and data sheet check lists in vaccine manufacture and testing.

Samples of these are included as annexes of this report.

DISCUSSION

Staff trained showed alot of enthusiasm and co-operation. In the opinion of the consultant, the personnel are now better placed to execute the demonstrated techniques and to correctly interpret

the results of each activity. A joint meeting held with production and quality control staff during the last week of this mission was used to review the activities done and to clarify any issues that needed further elaboration. It was generally felt that from time to time CVL needs to arrange staff training both internally and externally by organising training visits by staff to established vaccine laboratories to gain more experiences in vaccine manufacture and testing processes.

A trained work force is certainly an asset especially when one considers the long term sustainability of vaccine manufacture in view of the current moves towards privatisation of veterinary services in Africa. Taking into account the already installed laboratory infrastructure and equipment CVL compares favourably with regional vaccine production laboratories like those in Ethiopia, Botswana, Cameroon and Kenya. However, to match those other laboratories in terms of range of products manufactured and thus increased revenue from sales of such products CVL would need to consider making additional vaccines like those for poultry e.g. New Castle disease vaccine. This would of course involve purchase of new equipment and training of one or two other people but in the in the long term it will enable CVL to fund its operations from revenue from vaccine sales. And then CVL has to aggressively market its products both nationally and regionally.

ANNEX I

PROPOSED FREEZE DRYING CYCLE FOR CBPP VACCINE AT CVL, BAMAKO

DAY 1

A. DISPENSING

1. Pool vaccine harvests from individual baloon culture flasks in large enough vessel

2. Add a volume of 20% sterile skimmed milk suspension so as to obtain 4% final concentration of the stabilizer. Mix the vaccine culture and skimmed milk by swirling and the by magnetic stirring so as to ensure a homogeneous suspension.

3. Dispense vaccine into freeze drying vials (2.5ml for 8ml vials or 3.5ml for 17ml vials)

NB: During pooling and dispensing vaccine should be kept cool e.g. by placing vaccine container on ice.

4. Appy rubber stoppers loosely onto filled vials and then hold filled vaccine trays in the chiller.

5. When the filling and application of rubber stoppers is complete load chilled vaccine onto precooled shelves of the lyophilising machine

B. FREEZING

6. Cool vaccine to -40°C

7. Hold vaccine at -40°C for 3 hours

8. Switch on the condenser and when the condenser temperature has fallen to at least -50°C (usually after about 20 minutes) switch on the vacuum pump.

9. Set shelf temperature at -30°C and hold the vaccine under these conditions overnight.

DAY 2

10. Uniformly raise the shelf temperature to 0°C over 10 hours e.g. by 3°C after every hour.

11. Hold the vaccine under this temperature overnight

DAY 3

12. Raise shelf temperature at 20°C uniformly over 6 hours and hold the vaccine at that temperature overnight

13. Raise shelf temperature to 30°C over two hours and hold for 2 hours

14. Stopper vaccine vials under vacuum

15. Unload vaccine from the lyophilizer, apply aluminium caps anto vaccine vials and keep the vaccine at -20°C while awaiting quality control testing or dispatch.

NB: At hourly intervals throughout this lyophilisation the following parameters should recorded:
- set shelf temperature
- product temperature of each shelf
- condenser temperature
- vacuum level
- time of raising shelf temperature
- any alterations on the above protocol

ANNEX II

CBPP VACCINE PRODUCTION AND QUALITY CONTROL DATA SHEETS

A. PRODUCTION RECORDS

I. INOCULUM

Date prepared	
Reference number	
Seed strain designation	
Passage level	
Duration of incubation	
Medium used	
Sterility test result	
Signature of operator and date	

II. BULK VACCINE CULTURE HARVESTS

Date of inoculation	
Number of vessels inoculated	
Medium used	
Volume of medium per vessel	
Duration of incubation	
Sterility test result of neat culture pool	
Sterility test result of skimmed milk	
Titre of vaccine pool + skimmed milk	
Signature of operator and date	

III. FINAL PRODUCT

Batch number	
Vial size used	
Filling volume per vial	
Stabilizer and concentration used	
Date of lyophilisation	
Duration of lyophilisation (Hours)	
Total number of vials	
Number of vials spoiled	
Quality control result	
Expiry date	
Signature of operator and date	

ANNEX III
CBPP VACCINE QUALITY CONTROL RESULTS SUMMARY
RECORD SHEET

Batch/Lot Number:
Manufacture date:
Expirydate:

TEST TYPE	RESULT (Tick One)
1. Vacuum	Pass/Fail/Not done
2. Sterility	Pass/Fail/Not done
3. Titre/dose	Pass/Fail/Not done
4. Identity (specify test used)	Pass/Fail/Not done
5. Innocuity -in mice -in guinea pigs	 Pass/Fail/Not done Pass/Fail/Not done
6. Safety in cattle	Pass/Fail/Not done
7. Potency test in cattle	Pass/Fail/Not done
8. % residual moisture	Pass/Fail/Not done
CONCLUSION	PASS/FAIL

Signature of officer responsible for QC: Date:

ANNEX IV

ADDITIONAL EQUIPMENT NEEDED FOR CBPP PRODUCTION AND QUALITY CONTROL LABORATORIES

A. PRODUCTION LABORATORY

1. pH meter
2. Pipette aid/controller
3. 40 Litre flask
4. Large refrigerator
5. Precision balance
6. Air conditioner

B. QUALITY CONTROL LABORATORY

1. Pipette aid/controller
2. Repetitive pipette e.g. Eppendorf multipette
3. Dessicator
4. Refrigerator

C. DISPENSING AREA

1. Large laminar air flow cabinet

D. FREEZE DRYING

1. Freeze dryer (for a total of 2 functional)

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