TERMINAL REPORT

(SEPTEMBER 2004 – NOVEMBER 2005)

SERVICE CONTRACT financed under the 8th European Development Fund - Project Nr 8/ACP/TPS 32

PAN-AFRICAN PROGRAMME FOR THE CONTROL OF EPIZOOTICS (PACE)

SATEC DEVELOPPEMENT INTERNATIONAL

BY JOSEPH LITAMOI (NOVEMBER 2005)

Acronyms and Abbreviations

AU	African Union
CBPP	Contagious bovine pleuropneumonia
GMP	Good Manufacturing Practices
CIRAD-EMVT	Centre international de recherche agricole pour le développement – Département Elevage et médecine vétérinaire tropicale
LANAVET	Laboratoire National Vétérinaire, Garoua, Cameroon
LCV	Laboratoire Central Vétérinaire, Bamako, Mali
ND	Newcastle disease
CVRL	Central Veterinary Research Laboratory, Khartoum, Sudan
CTA	Chief Technical Advisor
EC	European Commission
EDF	European Development Fund
GLP	Good Laboratory Practices
GTZ	German Technical Cooperation (Deutsche fur Technische Zusammenarbeit)
QA	Quality Assurance
QC	Quality Control
IBAR	Inter-African Bureau for Animal Resources
FAO	Food and Agriculture Organization of the United Nations
NVI	National Veterinary Institute, Debre Zeit, Ethiopia
OAU	Organisation of African Unity
OIE	Office International des Epizooties
PACE	Pan African Control of Epizootic Diseases
PANVAC	Pan African Veterinary Centre
PARC	Pan African Rinderpest Campaign
PPR	Peste des petits ruminants
REA	Department of Rural Economy and Agriculture of the AU Commission
EU	European Union
SATEC	SATEC Développment International
TCP	FAO Technical Cooperation programme
ToR	Terms of Reference
WHO	World health organization
WP	Work Plan
TCP	Technical cooperation Project of the FAO

1. EXECUTIVE SUMMARY

Following the signature of the of its headquarters agreement (Maputo, 28th July 2003) between the AU and Ethiopian Government, PANVAC was re-opened in February 2004 with the arrival at duty station of the Chief Technical Advisor (Veterinary Vaccine Specialist and Acting Director) provided by CIRAD-EMVT. Although it was planned that the Technical Assistant (CBPP Vaccine Diagnostic and Vaccine Specialist) to be provided by SATEC/GTZ-IS would commence duty at the same time, it was not until September 2004 that the latter assistant was actually mobilized. The PANVAC work plan was prepared by the Chief Technical Advisor and approved by AU-IBAR/PACE and the EU Delegation in Kenya.

An inventory of the assets handed over to PANVAC by the NVI (PANVAC Host Institute) showed that although most of the laboratory equipment seemed to be serviceable, all the office equipment needed to be totally replaced. However, as vaccine quality control testing and biological standardization demands the use of precisely functioning laboratory equipment and instruments, a more detailed examination and testing of the actual functional status of individual pieces of laboratory equipment was carried out. This exercise revealed that some equipment like the freeze dryer, water purification systems, the various pipetters and some freezers were faulty. Where possible attempts were made to revalidate the equipment. Similarly, stock taking of the stores of laboratory materials (especially media and chemicals) showed that all had exceeded their expiry life. Therefore a list of essential office and laboratory equipment, media, chemicals and biological raw materials was compiled and their price quotations obtained from potential overseas suppliers. However, the purchase of these items was not possible because the indicated budgetary support from PACE or AU is yet to be released. This impacted negatively on the ability of PANVAC to implement planned activities.

Twelve samples representing a similar number of vaccine batches were submitted to PANVAC for quality control testing on cost recovery basis. The vaccine batch samples were received from the following laboratories: LANAVET, Garoua, Cameroon (3 batches of CBPP and 2 of PPR vaccine), LCV, Bamako, Mali (5 batches of CBPP vaccine) and NVI, Debre Zeit, Ethiopia (2 batches of CBPP vaccine). Test methods were as described in the PANVAC Standard Operating Procedures for such vaccines. Based on conformity with OIE norms and recommendations, all vaccine batches from LANAVET and NVI were certified while three out of five vaccine batches from LCV met the minimum requirements.

An integral part of PANVAC's mandates is the promotion of biological standardization and quality control of veterinary biological products as a key requirement towards the achievement of harmonised vaccine production and quality control methods throughout Africa. Some elements of this mandate include the preparation, testing and maintenance of a common repository of certified reference biological products. In this regard new stocks of CBPP and PPR reference vaccines preparations were produced and tested. Results obtained show that both reference preparations passed the tests for vacuum, purity, identity and potency. For inter-laboratory comparative purposes, samples from the CBPP reference vaccine stock were tested for *in vitro* potency at four of PANVAC network laboratories and the values obtained in the mycoplasma

titrations were very similar to those obtained at PANVAC. Similarly a new stock of PPR vaccine seed was prepared and tested for quality with satisfactory results. Upon request some key certified biological materials (vaccine seeds, antisera and reference vaccine preparations) were supplied to a number of vaccine production laboratories.

Collaborative experiments between PANVAC and the NVI on the comparative evaluation of the safety and efficacy of a regular and "Xerovac" Newcastle disease (ND) vaccines were successfully concluded. An analysis of the results obtained showed that the serological response profiles of the vaccinated chicks were similar for the two types of vaccines. The unvaccinated control chicks were negative for ND antibody until the time of challenge. When subjected to an intramuscular virulent challenge at three weeks post-immunisation, all vaccinated chicks withstood the infection while 60% of the unvaccinated control birds died of ND.

Another key mandate of the current PANVAC work plan was to assist the African Union in the PANVAC institutionalisation process. In this regard the following actions were taken:

- A draft PANVAC Constitution defining governance, executive bodies and mandates for this institution was prepared and forwarded to AU for consideration;

- A PANVAC Structure was proposed to the AU Department of Rural Economy and Agriculture and subsequently approved by the AU Executive Council.

- It was recognized that the PANVAC financial sustainability would depend ultimately on the extent to which it would be able to generate revenue from its diversified range of activities and to attract external support. Therefore a concept paper on PANVAC Funding Policy and Actions was formulated and forwarded to the AU Department of Rural Economy and Agriculture for consideration and action.

- Similarly, and with the same objective, another Concept note on the development of AU/PANVAC as an African centre of excellence in veterinary vaccine science was prepared and submitted to AU.

Actions in support of capacity building in some network laboratories were undertaken and these included hosting of team of five senior laboratory personnel from the National Veterinary Research Institute (NVRI), Vom, Nigeria and a team of two senior Veterinarians from Veterinary Vaccines Production Centre (VVPC), Nairobi, Kenya. The purpose of the visit was to map out areas of collaboration between PANVAC and NVI on one hand and VVPC on the other hand. Following lengthy deliberations it was felt that scientific collaborations on areas of mutual interest are best implemented through exchange of letters of agreement or memoranda of understanding. A Veterinarian from a diagnostic laboratory in Austria made a one week scientific visit to PANVAC to discuss matters pertaining production of autogenous vaccines for porcine.

One Veterinarian and one Technician from the Central Veterinary Research Laboratory (CVRL), Khartoum, Sudan undertook a ten-day training course on the quality control testing of CBPP vaccine at PANVAC. This was followed by one-month mission by a PANVAC scientist to CVRL for further in situ training in the production and quality control of CBPP vaccines. A technical backstopping on the production of thermostable PPR and Newcastle disease vaccines at LANAVET, Cameroon was also undertaken by PANVAC's CTA. All the latter three missions were sponsored by FAO TCPs in the respective countries.

The preparation of a manual of master formulae and processing instructions for PPR vaccine production and quality control is now complete and is for final editing and publication. The adoption of the methods and procedures described in the manual by vaccine production laboratories in Africa will contribute immensely to the harmonization of this vaccine production and quality control test methods in Africa.

2. TERMS OF REFERENCE

CBPP Diagnostic and Vaccine Specialist

Post level: P4

Location: PANVAC, Debre Zeit, Ethiopia

Immediate Supervisor: PANVAC Chief Technical Advisor

Major duties and responsibilities

The expert will be responsible for the technical activities relating to the diagnosis of CBPP and the quality control of CBPP vaccine. He/She will work as advisor to the Director of the AU/IBAR under the supervision of the PANVAC expert in veterinary vaccines to whom he will report.

The CBPP Diagnostic and Vaccine Specialist will:

1. Implement the laboratory activities for the quality control testing of CBPP vaccine and other priority vaccines, according to international standards;
2. Implement the laboratory activities for the diagnosis of CBPP and the evaluation of diagnostic methods;
3. Participate in the preparation and/or maintenance of a PANVAC repository of seed material, cell cultures, quality control standards and key reference reagents for use in CBPP vaccine quality control;
4. Assist in the preparation of PANVAC Quality Manual;
6. Participate in the implementation and monitoring of Good Laboratory Practices (GLP) and quality assurance principles in PANVAC's laboratories;
7. Assist in the production of Standard Operating Procedures and other instruction manuals for use in the production and quality control of CBPP vaccine and other priority vaccines for Africa;

8. Assist in the preparation of Manufacturing instruction manuals (Master Formulae), Standard Operating Procedures Manuals and other technical manuals for priority vaccines;
9. Contribute to the promotion of the regional production, standardization and distribution of biologics used for animal disease diagnosis and surveillance;
10. Contribute to PANVAC's communication programme;
11. Participate into PANVAC training activities;
12. Participate in implementation of PANVAC biologics information collection and dissemination;
13. Assist the Director in the preparation of PANVAC's work plans and reports;
14. Perform other related duties as required by the Director of PANVAC.

Educational qualifications: Candidates must hold a degree in veterinary medicine and postgraduate degrees in the field of bacteriology

Work experience: The incumbent should have a minimum of 10-year experience in veterinary vaccines production and quality control. He/She should have established expertise in modern vaccine production technologies, a sound knowledge of the requirements for Quality Assurance and Good Manufacturing Practices as applied to vaccine manufacture. Previous working experience at PANVAC would constitute a distinct advantage. He/She should have a competence in the use of a variety of computer software packages and proficiency in two of the African Union working languages

3. INTRODUCTION

Background

Following the resurgence of rinderpest in Africa in the 1980's and the subsequent launching of Pan African Rinderpest Campaign (PARC), an Expert Consultative meeting convened by FAO in 1984 noted that there was quantitative shortage of rinderpest vaccine supply, wide discrepancy in the quality of the vaccines and wide variation of quality control methods. Since the PARC programme was to hinge on vaccination and hence availability of good quality rinderpest vaccine was essential to the success of the programme, the meeting urged all rinderpest vaccine producing laboratories to participate in an international and independent vaccine quality control scheme. In furtherance of this and using its TCP resources, FAO established two Regional Veterinary Vaccine Quality Control and Training Centres at Debre Zeit (Ethiopia) and Dakar (Senegal). Between 1988 and 1992 the two centres were funded by the UNDP as a single project, and became known as the Pan African Veterinary Vaccine Centre (PANVAC) with the OAU/IBAR and FAO as the implementing Agency and executing agencies respectively. In 1993, the two centres were merged at one site in Debre Zeit due to the lack of immediate funds. Thereafter, the funding of the Centre was successively assured by the FAO (December 1995 - June 1996), the European Commission (July 1996- June 2000 for the Quality control Component) and by Japan (April 1997-March 2002 for the Developmental and standardization Component).

The Centre's achievements have been well appreciated and recognized by various evaluation and review teams, consultants, beneficiary laboratories and governments who reported that PANVAC's activities have resulted in a **significant improvement in**

the quality of rinderpest vaccine and other priority vaccines produced in Africa. It was to strengthen these achievements in the interest of Africa that the 67th ordinary session of the OAU Council of Ministers (Addis Ababa, 23 to 27 February 1998) decided to elevate PANVAC to the level of an OAU Specialized Agency.

In 1999, following the recommendation of the OIE evaluation mission of PANVAC to fund the Centre for a 5-year period (September 1997), the European Development Fund decided to support the Centre in the framework of the Pan African Programme for the control of epizootics (PACE, REG/500/005), in order to give time to the then OAU to achieve the institutionalisation of PANVAC.

PANVAC technical execution was tendered in March 2000 and awarded to CIRAD-EMVT. The contract signed, on 15th June 2000, comprised the provision of two technical assistants (one expert in veterinary vaccines for 30m/m and one CBPP diagnostics and vaccine expert for 12m/m). The posting of the two experts could not take place at the time, due to the absence of a PANVAC Host country and Host institution agreement between OAU and Ethiopian authorities (previously PANVAC had been executed as a FAO project under the umbrella of FAO Representation in Ethiopia) so that the quality control activities of PANVAC were suspended in July 2000 and the developmental activities in June 2002. The signature of PANVAC's headquarters agreement (28th July 2003) and the financial support of the European Commission (EC), through the PACE programme, permitted the reopening of the Centre in February 2004 with the arrival of the Technical Assistant provided by CIRAD-EMVT (as Chief Technical Advisor – CTA- of PANVAC). PANVAC was officially launched, as an AU Regional Centre, in Debre Zeit within the compound of its hosting institution, the National Veterinary Institute (NVI) of Ethiopia (12 March 2004).

The provision of the CBPP Diagnostic and Vaccine Technical Assistant was secured through contractual arrangements between AU-IBAR on one hand and GTZ-IS/SATEC on the other hand.

Implementation arrangements

The PACE programme (of which PANVAC is a component) is funded by a grant from the 8th European Development Fund and the Agency responsible for its implementation is the Inter-African Bureau for Animal Resources (AU-IBAR). The activities of PACE are coordinated by a Programme Coordinator who was appointed by the Director, AU-IBAR. IBAR made contractual arrangements with organisations like GTZ-IS/SATEC and CIRAD-EMVT for provision of technical assistance to PACE.

Within the framework of the PACE programme the PANVAC CTA, in close collaboration with the PACE Coordinator, was responsible for the overall management of PANVAC, functions that were supposed to be progressively handed over to the PANVAC Director to be recruited by the AU. The CBPP Diagnostic and Vaccine Specialist who was provided through a SATEC contract in cooperation with GTZ-IS worked under the CTA as a Freelance Consultant. Although expected to enter duty in February 2004, for some unclear reasons it was only possible for the CBPP expert to be mobilized at the beginning of September for an initial two months which was later extended by one year and then by another 2 months.

The PANVAC programme was implemented on the basis of a Work Plan and cost estimates developed by the CTA in close collaboration with the PACE Coordinator and endorsed by the Director of AU/IBAR (as PACE Regional Authorizing Officer) and the Delegate of the European Commission Delegation in Nairobi (EC). The financing of the PANVAC Work Programme was foreseen in the PACE Financing Agreement over the funds of the Regional Component.

The contribution of the host country government, the Government of the Federal Democratic of Ethiopia, included the provision of laboratory and office spaces, access to utilities (electricity and water) and other facilities usually granted to similar international organizations (e.g. duty free privilege, exemption from search, permission to operate a foreign bank account etc...). The National Veterinary Institute (NVI) is the hosting institution of PANVAC.

Mandates

According to the current work plan the specific objectives of PANVAC are to:

(1). Provide international quality control testing and certification for Africa's priority vaccines (on a cost-shared basis)
(2). Promote biological standardization and quality control of veterinary products
(3). Promote the transfer of appropriate veterinary vaccine technologies to African laboratories including adaptation to suit local conditions
(4). Provide training and technical backstopping services to the national vaccine production laboratories in Africa
(5). Establish a regional facility for the production and standardization of reagents for animal disease diagnosis and surveillance
(6). Assist in the creation of optimal conditions to ensure viability and sustainability of the centre as a Specialized Agency of the AU

Due to time and resource constraints three of the above mandates were identified as priority immediate objectives to be implemented during the current transitional phase of PANVAC. These short term objectives as approved in the work plan that was submitted to the management of PACE programme were to:

- **Restore PANVAC's veterinary vaccine quality control activities,**
- **Resume the standardisation of veterinary biological products and the harmonization of veterinary vaccines quality control techniques and**
- **Assist African Union in PANVAC institutionalisation process**

This terminal consultancy report presents an outline of activities implemented and the achievements obtained between September 2004 and November 2005 in relation to the approved immediate objectives for PANVAC. It was prepared as part of the contractual obligations entered into and agreed with SATEC and GTZ-IS on one hand and the Technical Assistant/Consultant on the other hand.

4. MAIN TASKS PERFORMED DURING THE PERIOD

4.1 Appraisal of status of laboratory facilities, equipment and supplies

PANVAC activities were suspended since mid-2000 due to administrative problems. All PANVAC assets were therefore handed over to the NVI by FAO when the latter ceased to be the Executing Agency for PANVAC.

The centre was re-opened in February 2004 with arrival of the CTA – Acting Director of PANVAC- who was recruited by CIRAD-EMVT. Subsequently an inventory of the assets handed over to PANVAC by the National Veterinary Institute of Ethiopia (PANVAC Host Institute) showed that although most of the laboratory equipment was serviceable, the office equipment would need to be totally replaced. As vaccine quality control testing demands the use of precise and properly functioning laboratory equipment and instruments, a more detailed examination and testing of the functional status of individual pieces of laboratory equipment was carried out. This exercise revealed that some equipment like the Edwards Supermodulyo 12K freeze dryer had a faulty vacuum system. This has now been rectified with the help of NVI and the machine has been successfully used to produce a stock of PPR vaccine seed as well as PPR and CBPP reference vaccine preparations. Similarly, the various types of pipettors were tested and most of them were found to be defective. It was possible to re-calibrate and validate a few but the majority of them were found unserviceable and new replacements will therefore have to be procured. The installed water purification and distillation machines are either not working or require pre-treatment and reverse osmosis cartridges. Most of the refrigerators, freezers and incubators appear to be in good working condition but the accuracy of their readings would still need to be validated and additional ones are required to cater for expected demand for more storage space for the various types of biologicals being maintained at and/or those that will be prepared by PANVAC. Similarly, all laboratory materials (especially media and chemicals) had exceeded their expiry life and fresh stocks should have to be purchased. Up to now no purchase has materialized due to lack of operating funds.

Recommendation

The inspection of equipment and supplies as indicated above strongly suggests the need for fresh purchase of some laboratory and office equipment, chemicals, media, reagents and other consumables. An indicative list of the minimum purchases needed was drawn up and included in the inception report of both the CTA and the reporting officer as well in the work plan submitted to PACE programme. Price quotations from prospective suppliers have been obtained. However, the finances to permit the purchases during this consultancy mission were never disbursed. It is therefore strongly recommended that sufficient funds should be urgently sought and availed to PANVAC by the Commission of the African Union if the objective of developing PANVAC into a Centre of excellence in vaccine science is to be realized.

4.2 Independent Vaccine Quality Control testing and Certification

Twelve samples representing a similar number of vaccine batches were submitted to PANVAC for quality control (QC) testing on cost recovery basis. The vaccine batch samples were received from the following laboratories: LANAVET, Garoua, Cameroon (two batches each of CBPP and three of PPR vaccine), LCV, Bamako, Mali (5 batches of CBPP vaccine) and NVI, Debre Zeit, Ethiopia (2 batches of CBPP vaccine).

Each batch of vaccine was tested for vacuum, sterility (purity), identity, adventitious agents and mycoplasma (PPR vaccines) and potency. Test methods and procedures were carried out as described in the PANVAC Standard Operating Procedures for the respective vaccines. The results obtained and conclusions made were as summarised in tables 1 – 4 below:

Table 1: QC test results of LANAVET PPR Vaccines

Test type	Vaccine Batch No. And results		
	Batch 016C	Batch 017C	Batch 018C
Seed strain	PPRV75/1	PPRV75/1	PPRV75/1
Doses/vial	50	50	50
Vacuum	Pass	Pass	Pass
Potency (in vitro)	Log_{10} 4.56 $TCID_{50}$/vial	Log_{10} 4.70 $TCID_{50}$/vial	Log_{10} 4.42 $TCID_{50}$/vial
Titre/dose	Log_{10} 2.86 $TCID_{50}$	Log_{10} 3.0 $TCID_{50}$	Log_{10} 2.72 $TCID_{50}$
Sterility	No bacterial or fungal contamination detected	No bacterial or fungal contamination detected	No bacterial or fungal contamination detected
Mycoplasma	None detected	None detected	None detected
Identity	Pass	Pass	Pass
Adventitious agents	None detected	None detected	None detected

Table 2: QC results of LANAVET CBPP Vaccines

Test type	Batch 024N	Batch 023N
Seed strain	T1SR	T1/44
Doses/vial	100	50
Vacuum	Pass	Pass
Sterility	No evidence of bacterial or fungal contamination	No evidence of bacterial or fungal contamination
Mycoplasma content	Log_{10} 9.35 CCU_{50}/vial	Log_{10} 9.70 CCU_{50}/vial
Titre/dose	Log_{10} 7.35 CCU_{50}	Log_{10} 8.00CCU_{50}/ml
Identity - Growth inhibition	Pass	Pass
Identity – Agar gel immundiffusion	Pass	Pass
Identity – Streptomycin resistance	Resistant	Partially resistant
Identity - Colony morphology	Typical	Typical
Sensitivity to digitonin	Sensitive	Sensitive

Table 3: QC results of LCV CBPP Vaccines

Test type	Batch 275	Batch 286	Batch 298	Batch 299	Batch 300
Seed strain		T1SR	T1SR	T1SR	T1/44
Doses/vial	50	50	50	50	50
Vacuum	Pass	Pass	Pass	Pass	Pass
Sterility	No evidence of bacterial or fungal contamination	No evidence of bacterial or fungal contamination	No evidence of bacterial or fungal contamination	No evidence of bacterial or fungal contamination	Bacterial and fungal contamination detected
Mycoplasma content	Log_{10} 5.65 CCU_{50}/vial	Log_{10} 9.55 CCU_{50}/vial	Log_{10} 9.43 CCU_{50}/vial	Log_{10} 9.23 CCU_{50}/vial	Log_{10} 9.33 CCU_{50}/vial
Titre/dose	Log_{10} 3.95 CCU_{50}	Log_{10} 7.85CCU_{50}	Log_{10} 7.73CCU_{50}	Log_{10} 7.53CCU_{50}	Log_{10} 7.63CCU_{50}
Identity - Growth inhibition	Pass	Pass	Pass	Pass	Pass
Identity – Agar gel immundiffusion	Pass	Pass	Pass	Pass	Pass
Identity – Streptomycin resistance	Resistant	Resistant	Resistant	Resistant	Sensitive
Identity - Colony morphology	Typical	Typical	Typical	Typical	Typical
Sensitivity to digitonin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

Table 4: QC results of NVI CBPP Vaccines

Test type	Batch PL2/05	Batch PL3/05
Seed strain	T1/44	T1/44
Doses/vial	100	100
Vacuum	Pass	Pass
Sterility	No evidence of bacterial or fungal contamination	No evidence of bacterial or fungal contamination
Mycoplasma content	Log_{10} 9.57 CCU_{50}/vial	Log_{10} 9.57CCU_{50}/vial
Titre/dose	Log_{10} 7.57 CCU_{50}	Log_{10} 7.57CCU_{50}/ml
Identity - Growth inhibition	Pass	Pass
Identity – Agar gel immundiffusion	Pass	Pass
Identity – Streptomycin resistance	Sensitive	Sensitive
Identity - Colony morphology	Typical	Typical
Sensitivity to digitonin	Sensitive	Sensitive

On the basis of the QC results obtained all PPR and CBPP vaccine batches that were submitted by LANAVET were certified as having met the minimum OIE requirements for such vaccines. Similarly both CBPP vaccine batches from NVI were certified. Four out of five (80%) CBPP vaccine batches from LCV were certified. One batch (batch 300) failed on account of bacterial and fungal contamination.

Observations and Recommendation

The volume of vaccine batches received for PANVAC QC testing and certification is far below the expectations. It has been noticed that the vaccine manufacturers, whose participation in PANVAC vaccine quality control scheme is on a voluntary basis, are inclined to submit only vaccine batches destined for export markets. The low level of vaccine submission to PANVAC could also be traced to lack of funding that prevented PANVAC to hold the 3rd Meeting of Directors of Vaccine manufacturing laboratories (to get the commitment of PANVAC's first beneficiaries and arrive at a common view on cost of the services provided by PANVAC). For the same reason it was not possible to undertake communication/sensitisation activities towards amongst the Region's National Biologics Quality Control Authorities on benefits of the use of quality certified vaccines.

It is therefore recommended that, if PANVAC activities and achievements are to be sustainable, that sufficient funding should be availed and mechanisms through which the vaccine certification programme could be enhanced. Some of the actions that ought to be taken in this respect could include holding of meetings of Directors of Vaccine Production laboratories and National Quality Control Authorities to secure their commitments to agree to submit to PANVAC certification all vaccines destined for

national vaccination programmes. To win further confidence, competent technical personnel should also be recruited and posted to PANVAC.

4.3 Maintenance and/or preparation of repository of key biologicals

Following the conclusion of the taking of a physical inventory of the Reference biologicals currently maintained in the PANVAC repository, testing of some the key materials was undertaken. The tests so far carried have established that the state of the Vero cells in both the Master and Working Cell banks is satisfactory. Stocks from tested samples have, on request, been supplied to JOVAC laboratory, Jordan.

In vitro Quality Control (QC) testing of the newly prepared stock of PPR vaccine seed was accomplished. The seed has been shown to be free from bacterial, fungal and mycoplasma contamination. It was also tested for freedom from adventitious/ extraneous agents with satisfactory results. The virus titre of the seed stock was found to be Log_{10} 5.5 $TCID_{50}$

The CBPP hyperimmune sera being maintained in the PANVAC repository was tested for its mycoplasma growth-inhibiting and immunoprecipitating properties. The results obtained confirmed the potency of the serum stock.

Similarly the PPR reference vaccine preparation was tested and its mean titre was found to be Log_{10} 5.0 $TCID_{50}$. However, because the number of vaccine vials remaining is too low, a new stock had to be produced and validated before use in routine vaccine quality control.

CBPP vaccine seeds strains T1SR and T1/44 that were prepared in 1996 and 1997 respectively were found to be still viable albeit with reduced titres.

The mycoplasma content of CBPP reference vaccine preparations was found to be too low and a new stock had to be prepared.

Recommendation

All biologicals maintained in the PANVAC repository that have not been re-tested should be re-validated. The range of such reference materials should also be further diversified to include all vaccines for the priority animal diseases in Africa.

4.4 Production and testing of CBPP and PPR Reference Vaccine Preparations

An integral part of PANVAC's mandates is the promotion of biological standardization and control of veterinary biological products as a key requirement towards the achievement of harmonized priority veterinary vaccine production and quality control methods throughout Africa. Some key elements of this mandate include the preparation, testing and maintenance of a common repository of certified reference biologicals. In this regard new stocks of CBPP and PPR reference vaccines preparations were produced and tested. A new stock of PPR vaccine virus seed lot was also prepared and tested. Results obtained show that both reference preparations

and seed lot passed the tests for vacuum, purity, identity and potency. For inter-laboratory comparative purposes samples from the reference vaccine stocks were tested for *in vitro* potency at some selected PANVAC network laboratories. The procedures for the production and quality control for these reference biologicals is summarized below. Other actions taken in the promotion of adoption of harmonized vaccine production and testing methods included the preparation of a manual for PPR vaccine master production formulae (MF) and standard operating procedures (SOP).

4.4.1 CBPP Reference Vaccine production

Gourlay medium that had been previously prepared and tested for suitability was used in the production of the CBPP reference vaccine. A vial of freeze dried *Mycoplasma mycoides* subsp. *mycoides* (SC) vaccine seed strain T1/44 from the PANVAC repository of certified biologicals was reconstituted in sterile distilled water and inoculated into Gourlay medium. Inoculated medium was then incubated at 37^0C until sufficient mycoplasma growth (48 hours) was obtained as judged by visual examination of culture turbidity and pH measurement. The inoculum was tested for purity by Gram stain and then used to prepare bulk vaccine culture by infecting Gourlay medium at a ratio of one part inoculum to 9 parts medium. Inoculated bulk culture medium was incubated stationary at 37^0C for 44 hours when antigen harvest was made. As free drying stabilizer , a 10% sterile skimmed milk suspension was added into the vaccine harvest so as to obtain a final stabilizer concentration of 4%. Stabilizer and vaccine mixture was homogenized and dispensed into 5ml freeze-drying vials at a filling volume of 1.5ml/vial. Rubber stoppers were loosely applied to filled vials that were then loaded on to the shelves of an Edwards Supermodulyo freeze dryer ready for lyophilisation. The lyophilisation cycle used comprised a total of 68 hours and consisted of 3 hours freezing, 28 hours primary drying and 37 hours secondary drying. The freeze-drying was successfully executed and 800 vials of the vaccine are now available at PANVAC.

4.4.2 Vaccine quality control

Prior to being adopted for use as a reference in vaccine QC testing the vaccine preparation was subjected to the full complement of the required in vitro tests both at PANVAC and at some its network laboratories. The results of the QC tests were as summarised in table 5 below

Table 5: Quality control results of CBPP reference vaccine (Batch PAN/CBPP/01/05))

Test type	Result
Visual appraisal	Well formed freeze dried cake
Vacuum	Pass
Sterility	No evidence of bacterial and/or fungal contamination
Potency (*in vitro*) at PANVAC	$Log_{10}9.50CCU_{50}$ /vial
Potency (*in vitro*) at NVI	$Log_{10}9.36CCU_{50}$ /vial

Potency (*in vitro*) at LCV	$Log_{10}9.65CCU_{50}$ /vial
Potency (*in vitro*) at CVRL	On going
Potency (*in vitro*) at LANAVET	On going
Identity (Growth inhibition)	Pass
Identity (Agar gel immunodiffusion)	Pass
Identity (Colony characteristics)	Typical
Identity (Biochemical characteristics)	Pass
Streptomycin resistance	Partial
Sensitivity to Digitonin	Sensitive

4.4.3 Production of PPR Reference Vaccine Preparation

The requisite Vero cell line (PPR virus substrate), cell culture medium and other solutions were pre-tested and shown to be suitable for use in vaccine production. The Vero cell cultures were scaled-up by a three-way split of confluent monolayers. Cell culture suspensions were inoculated with PPR vaccine seed virus (strain PPRV75/1) at a multiplicity of infection (MOI) of 0.001. Infected cells and uninfected controls were incubated at 37^0C in a CO_2 incubator. On day 3 of incubation (first appearance of virus cytopathic effects - cpe) cell culture fluid was decanted and fresh medium added into each culture flask. Development of virus cpe was monitored microscopically every day. When cpe was estimated to be about 80% samples were collected and the cell culture flasks were removed from incubation and then stored frozen at -20^0C. Quality control tests on liquid vaccine harvest samples were satisfactory meaning that it could pass to the next stage of processing i.e. freeze drying.

Frozen liquid PPR vaccine harvests were subjected to two freeze-thaw cycles and pooled. Equal portions (v/v) of the thawed vaccine and 5% Lactalbumin hydrolysate/10% Sucrose were mixed. 1ml of the mixture was dispensed into 5ml freeze drying vials, loaded onto freeze dryer shelves and then subjected to a lyophilisation cycle that consisted of 3 hours freezing, 28 hours primary dehydration and 30 hours of secondary freeze-drying or desorption. A total of 450 vials were successfully freeze-dried.

4.4.4 PPR Reference Vaccine quality control

Quality control test results on the freeze-dried PPR vaccine reference preparation as obtained at PANVAC were as shown in table 6 below

Table 6: QC results on reference PPR vaccine preparation (Batch PPR02/50)

Test type	Result
Vacuum	Pass
Sterility	No evidence of bacterial or fungal contamination
Mycoplasma	Pass

Virus content	$Log_{10}5.5TCID_{50}/ml$
Identity	PPR virus identity confirmed by sero-neutralisation
Adventitious/extraneous agents	Pass
Residual moisture content	2.65%
Thermostability	Not done

NB: Subject to availability of funds samples from vaccine prepared will be send to PANVAC network laboratories for comparative quality control tests.

Recommendation

Necessary arrangements should be made to ensure that the produced reference vaccine preparations are tested by the entire network of PANVAC laboratories as one of the first steps towards harmonizing for the production and testing of vaccines

4.4.5 Preparation and QC testing of PPR Vaccine seed stock

A physical inventory of biologicals maintained at the PANVAC repository of reference materials revealed that the master seed stock for PPR vaccine was almost depleted. It was therefore urgent for a fresh stock to be prepared. Vero cells were inoculated with PPR vaccine virus at a multiplicity of infection of 0.001 followed by incubation at 37^0C. When about 80% of the cells showed evidence of virus cytopathic effects culture harvest was made. Samples of liquid virus harvest was tested for sterility and virus content, the remaining harvest was stored at -30^0C. Thawed (two freeze-thaw cycles) vaccine was mixed with equal volume of 5% lactalbumin hydrolysate-10% sucrose stabilizer, dispensed into freeze drying vials (1ml/vial) and then lyophilized for about 50hours. The freeze dried vaccine (300 vials) has been subjected to the full complement of in vitro QC tests.

In vitro Quality Control (QC) testing of the newly prepared stock of PPR vaccine seed has the seed to be free from bacterial, fungal and mycoplasma contamination. It was also tested for identity and freedom from adventitious/extraneous agents with satisfactory results. The virus titre of the seed stock was found to be Log_{10} 5.5 $TCID_{50}$

4.5 Comparison of the efficacy of freeze dried and Xerovac Newcastle disease vaccines

Newcastle disease is a devastating cause of huge economic losses in poultry farming whenever it infects susceptible chicken. Current vaccines for the prevention of Newcastle disease are freeze dried products and due to their heat lability they must be maintained under a cold chain right from the time of production to use in the field. This adds to the cost of the vaccine

Experiments were therefore carried out aimed at comparing the efficacy of freeze-dried and Xerovac Newcastle. The Xerovac process is a novel technique that has

been shown to yield heat tolerant live viral vaccines. This means that vaccines prepared using this method would require minimal refrigeration. The details of the experiments and results obtained were as summarised here below

Materials and methods

Chicken: Day old chicks were bought from Kombolcha poultry farm and then transported by truck to NVI, Debre Zeit, where they were kept in quarantine for 30 days with close observation. Each animal was identified by the application of a numbered tag.

Pre-inoculation serology: At the end of the quarantine period all the chicken were bled for serum. Serum samples were tested for Newcastle antibodies by the haemagglutination inhibition (HI) test

Vaccination: 14 birds were vaccinated with Newcastle Lasota Clone 30 Xerovac vaccine while 15 received a similar freeze-dried preparation. 10 chicken were maintained as unvaccinated controls

Challenge: Immediately before challenge all vaccinated and control birds were bled for serum. Seroconversation and titres of Newcastle disease virus antibody were determined by Haemagglutination-inhibition tests. The chicken were then subjected to virulent challenge by intramuscular inoculation of a Vero-cell propagated local virulent isolate of Newcastle disease virus (0.5ml of the local virulent was inoculated into each bird via the intramuscular route). The virus content of this culture was estimated to be $Log_{10}6.2TCID_{50}$/ml.

Post challenge monitoring:

Birds under challenge were observed daily for one month. Particular attention was paid to appearance of clinical signs suggestive of Newcastle disease. Postmortem examination was performed on all chicken that died and those that were killed *in extremis*

Results

Serum samples collected from all chicken prior to vaccination were negative for Newcastle haemagglutinating antibody. Similarly, all birds remained healthy until the time of challenge. The post-inoculation serological response profiles of the vaccinated chicks were similar for the two types of vaccines. The unvaccinated control chicks were negative for ND antibody until the time of challenge. All vaccinated chicks withstood virulent challenge that was administered three weeks following immunisation while 60% of the control birds died of ND.

Table 7: Vaccination-challenge results of experimental chicken (Table to redo?)

Group	Tag No.		Prevaccination HI titre	Prechallenge HI titre	Sick/Dead	PM lesions
Group 1: Inoculated with La Sota clone 30xerovac vaccine	1		0	1/512	0	
	2		0	1/128	0	
	3		0	1/64	0	
	4		0	1/32	0	
	5		0	1/32	0	
	6		0	1/64	0	
	7		0	1/64	0	
	8		0	1/32	0	
	9		0	1/64	0	
	10		0	1/64	0	
	11		0	1/16	0	
	12		0	1/16	0	
	13		0	¼	0	
	14		0	1/16	0	
Group 2: Inoculated with La sota clone 30 freeze dried vaccine	1		0	1/256	0	
	2		0	1/128	0	
	3		0	1/64	0	
	4		0	1/32	0	
	5		0	1/32	0	
	6		0	1/64	0	
	7		0	1/64	0	
	8		0	1/256	0	
	9		0	1/32	0	
	10		0	1/64	0	
	11		0	1/64	0	
	12		0	1/32	0	
	13		0	1/64	0	
	14		0	1/64	0	
	15		0	1/16	0	
Group 3: Uninoculated controls	1	0	0	6/2/05		
	2	0	0	8/2/05		
	3	0	0	10/2/05	Intestinal haemmorhages	
	4	0	0	10/2/05		
	5	0	0	13/2/05		
	6	0	0	15/2/05		
	7	0	0	0		
	8	0	0	0		
	9	0	0	0		
	10	0	0	0		

HI=Haemagglutination inhibition ; PM=Post mortem

The results obtained demonstrate that the two vaccine preparations were safe and gave a similar level of protection against Newcastle virulent challenge three weeks after immunisation. However, previous heat resistance tests had shown the Xerovac vaccine to be more heat tolerant than its freeze-dried counterpart.

Recommendation

The Xerovac technology would therefore seem to offer a method through which relatively heat stable Newcastle vaccines can be produced. Such heat tolerant vaccines would be suitable for use in rural areas of Africa where refrigeration facilities for vaccine storage may not always be available.

4.6 Training and Technical Support Services

The following actions were taken in support of capacity building of vaccine production laboratories:

Two staff members of the Central Veterinary Research Laboratory (CVRL), Khartoum, Sudan were sponsored by FAO to undertake a 10-day training at PANVAC on CBPP vaccine quality control. The trainees received a hands on training in such fields as enumeration of mycoplasmas, tests for vacuum, identity tests for *Mycoplasma mycoides* subsp. *mycoides* (small colony), biochemical characterization as well as detection of bacterial and fungal contamination. They also had a chance to tour vaccine production facilities of the NVI.

At the request of CVRL and FAO and with the concurrence of SATEC, GTZ, AU, IBAR and PANVAC, the CBPP Diagnostic and Vaccine Specialist undertook a one month's duty travel to the CVRL, Khartoum. The mission was was sponsored by FAO and the purpose was to offer technical assistance to a TCP project currently being implemented at CVRL. The specific terms of reference for the assignment were to:

- Assist in identifying items of materials and equipment for purchase
- Assist in the reorganization of CBPP vaccine production unit at CVRL in compliance with basic GLP/GMP requirements
- Set up, establish and test CBPP Vaccine Reference (master) bank to be used by CVRL as recommended by OIE
- Prepare some lots of CBPP vaccine batches
- Ascertain and assure quality control of lots produced
- Define and conduct on-the-job (in situ) training for technical personnel in the CBPP vaccine unit
- Participate in training workshops for the surveillance and control CBPP/CCPP in Sudan
- Perform any other related duties as required
- Prepare a report at the end of each mission and submit it to FAO for clearance and distribution

These tasks were successfully implemented as demonstrated in the cleared consultancy mission report, a copy of which was recently forwarded to SATEC, GTZ-IS, AU, PACE coordination and PANVAC.

A team of five senior laboratory personnel from the National Veterinary Research Institute (NVRI), Vom, Nigeria made a FAO-sponsored scientific study tour to PANVAC in November 2004. The purpose of the visit was to seek information on the current state of technological play and trends in vaccine quality assurance. Valuable discussions were held and views exchanged in this respect. The scientists availed themselves of the opportunity to tour the PANVAC laboratory facilities and those of the National Veterinary Institute of Ethiopia, which is making great strides in the implementation of quality management systems in its vaccine production operations. Upon return to Nigeria, enquiries have already been made by NVRI on the possibilities to send technical personnel from this laboratory for training at PANVAC.

Similarly two senior Veterinarians from Veterinary Vaccine Production Centre, Nairobi, Kenya visited PANVAC and the NVI. Various topics of mutual interest in the field of vaccine sciences were discussed. The need for closer collaboration between PANVAC and its network laboratories was emphasized and all agreed that implementation of identified areas of such collaborative efforts would be through an agreed memorandum of understanding.

A refrigeration and instrumentation technician from Laboratoire Central Veterinaire, Bamako, Mali visited PANVAC in December 2004 and was trained on the application of Xerovac technology in the production of heat resistant vaccines.

A Veterinarian from a Veterinary Diagnostic Laboratory at Vienna, Austria made a one-week study tour to PANVAC to seek scientific information and guidance on the production and quality control of autogenous vaccines against *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis in pigs.*

Recommendation

One of the major expectations from PANVAC is assistance in capacity building within the Region's veterinary vaccines manufacturers and regulatory authorities (National Quality control Authorities and their National Quality control Laboratories). It is therefore important to develop and implement a sound communication plan for PANVAC with a view to facilitating access of these institutions or organizations to training, technical support and information about veterinary vaccines and other biologics. This will also improve the demand on certified vaccine batches (at least for Africa's major priority vaccines) as emphasized by the various PACE Coordination meetings.

4.7 Supply of reference biologicals

As part of the efforts to harmonise vaccine production and quality control methods certified CBPP and PPR vaccine seeds as well hyperimmune PPR serum were supplied to LANAVET, Garoua, Cameroon. Similar types of biologicals were also supplied to National Veterinary Institute, Debre Zeit, Ethiopia.

On request CBPP (T1/44) vaccine seed and polyclonal antiserum was supplied to the Central Veterinary research Laboratory, Khartoum, Sudan.

JOVAC, Amman, Jordan, purchased Vero cells.

4.8 Assistance in the PANVAC institutionalization and sustainability process

Prior to 2000, the legal status of PANVAC had never been worked out and the Centre was operating as a project with a finite life span according to arrangements between the then OAU/IBAR, FAO, Governments and donor agencies. The **Headquarters agreement** signed on 8th July 2003 conferred to PANVAC the full status of an international organisation in Ethiopia. One of the objectives of this interim phase of PANVAC was also to assist the African Union Commission and AU/IBAR in the Centre's institutionalisation and sustainability processes.

The following documents were therefore prepared the project and submitted to the Department of Rural Economy and Agriculture for consideration by the AU Commission:

(a). Draft of the **PANVAC Constitution** was prepared and submitted to the Directorate of Rural Economy and Agriculture and AU-IBAR for consideration by the AU Commission. The draft proposed the composition, mandates and functions of governing and executive bodies

(b). Similarly a proposal of **PANVAC structure** was prepared and submitted to Department of Rural Economy and Agriculture of the AU. The draft which made proposals on the organogram, basic staffing and job descriptions was discussed and accepted by the Permanent Representatives' Council. It subsequently adopted by 6th Extraordinary Session of the Executive Council (6-7 December 2004, Addis Ababa).

(c) The draft of PANVAC's **Strategic Development Plan** (2004-2008) was submitted to AU/IBAR and AU/REA in May 2004. This proposal has been taken into account and included in the "Department of REA Strategic plan 2004-2007" that was submitted to Heads of States Summit in Addis Ababa in July 2004. At the request of the AU Commissioner for REA, this Strategic Plan has been translated into a **5-year project document** and re-submitted in July 2004.

(d). The **PANVAC financial sustainability** will depend ultimately on the extent to which it will be able to generate revenue from its diversified range of activities and to attract external support. Therefore, there is need for PANVAC to develop and implement a clear funds mobilization and allocation strategy. Such a strategy would allow the mobilization of complementary funds and other resources from sponsoring Governments, organizations or donor agencies for the full implementation of the PANVAC mission and objectives. Therefore a concept note on proposals for "**Funding policy & actions for PANVAC**" was prepared and submitted to DREA for consideration in February 2005. This concept note has to be discussed within AU before submission to potential donors.

(e). A concept note for the holding of a "**Third Pan African Meeting of Directors of Veterinary Vaccine Laboratories**" has been submitted to AU/IBAR for funding through the PACE programme (February 2005). It has to be recalled that only two such meetings have been so far organized in the Region: the first one in Nairobi (27-28 November 1990), Kenya and the second one in Dakar (6 – 8 July 1992), Senegal. The rationale for a third meeting is that on one hand veterinary vaccine producing laboratories are the first beneficiaries of the various services offered by PANVAC and on the other hand, the sustainability of PANVAC is intimately linked to the viability and well being of these vaccine manufacturers in Africa. Therefore, such meeting would be crucial to:

- Review the state of veterinary vaccines production, quality control, distribution and post-marketing monitoring in Africa;
- Discuss mechanisms for improving sustainability of laboratories that produce veterinary vaccines and other biologics;
- Promote the adoption and adherence to standards;
- Discuss PANVAC's strategic development plan with particular emphasis on the Centre's sustainability factors and on mechanisms to better capture expectations and feedback of its networked laboratories and the community;
- Review the state of veterinary laboratory registration, certification and accreditation in Africa

(f). At the request of the AU Commissioner for REA, the last draft submitted by the project in August 2005 was a "**Concept Paper for Development of the AU/PANVAC into a Regional Centre of Excellence for Veterinary Biologics in Africa**".

Recommendations

Now that AU is in the process of recruiting its own staff to run PANVAC the former should go further and approve PANVAC's Constitution to be followed by establishment and the convening of the first meeting of PANVAC's Governing bodies (Board of Trustees and Technical Advisory Committee). AU should also move fast to define and implement a clear resource mobilization to secure regular and relatively stable financial resources for PANVAC in order to operationalize mechanisms for governance and the management of PANVAC's programmes on a sustainable basis.

In order to strengthen all facets of its Strategic Plan, PANVAC will need to ***gather inputs*** *from the Region's laboratories and from a wide spectrum of key institutions, agencies and leading scientists for identifying key regional concerns and issues, to give guidelines for its future direction and assist in identifying opportunities for cooperation and collaboration. Therefore, it is highly advisable that the following consultations take place:*

- *A Meeting of* ***Directors of the Region's laboratories;***
- ***A Technical consultative meeting*** *with key institutions, agencies and leading scientists; and*
- ***A Resource mobilization meeting*** *with donor agencies.*

In addition to the posting of competent and sufficient staffing to PANVAC, it is essential that the operating funds should be rapidly be made available to permit the purchase of essential laboratory and office equipment and consumables as well as vehicles. The promised Euro 200,000 from the French Cooperation could be used for this purpose. The expected funds from the AU Commission could be mobilized for the supply of laboratory consumables, reagents and other materials.

5. MAIN DIFFICULTIES ENCOUNTERED IN PROJECT IMPLEMENTATION

The single biggest constraint faced during the implementation of project activities is the lack of funds, throughout these two years, to purchase necessary inputs (such as a vehicle, equipment, media, chemicals and laboratory consumables). Neither operation fund from PACE nor the expected budgetary allocation from AU solidarity fund has thus far been disbursed.

The consequence of this is that most planned activities were not implemented. Shortage of support personnel: The PANVAC project document has budgetary provisions for the employment of support staff such as laboratory technicians, laboratory assistant, secretary, driver and cleaner. However, these positions have not been filled because the necessary fund has not been availed.

6. ACKNOWLEDGEMENTS

The Reporting Officer wishes to thank SATEC, GTZ-IS, AU-IBAR and PACE Coordination for giving him an opportunity to offer his services at PANVAC. The cooperation of the NVI (PANVAC Host Institute) is also gratefully appreciated.