SADC SECRETARIAT **EUROPEAN DEVELOPMENT FUND**

Promotion of Regional Integration in the SADC Livestock Sector (PRINT Livestock Project)

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Report of a Mission to the Republic of Zambia Reinforcement of the capacity on Contagious Bovine Pleuropneumonia (CBPP) Diagnostics for SADC Veterinary Laboratories Network

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Dates: 25th June – 29th June 2007

Reinforcement of the capacity on Contagious Bovine Pleuropneumonia (CBPP) Diagnostics for SADC Veterinary Laboratories Network

PRINT PROJECT
Zambia 25th -29th June 2007

Objective of the workshop
To improve the capacity of each SADC Veterinary Laboratory that is involved in CBPP surveillance and diagnostic in CBPP infected countries, and those in CBPP-free countries which are at risk.

Organizer Institution
CIRAD UPR15 World reference Laboratory for CBPP for FAO, OIE reference Laboratory

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Program

Monday
Introduction, medium preparation, culture (liquid, solid media)
Tuesday
Serology, cELISA, pipetting, preparing CFT reagents
Wednesday
Complement fixation Test, Observation of MmmSC cultures, sample preparation for PCR
Thursday
PCR, agarose gel electrophoresis

Friday
General discussion on critical points, harmonized SOPs, round-robin organization

Specific objective of the workshop

Each CBPP diagnostic technique will be implemented in a demonstration with a focus on critical points that affect the quality of the results. These critical points will be discussed by the various participants of the workshop to ensure a list of recommendations to be implemented in the SADC participating veterinary laboratories before interlaboratory testing is organized. Recommendations for the definition of harmonized Standard Operating Procedures will also be issued.

These recommendations and their implementation will be reviewed during the second workshop organized at a later period (September 2007 in Tanzania) by PRINT

Critical points examined

The following critical points were taken for further deliberation and possible recommendations

1. **Media preparation**

a. Work under sterile conditions
b. Make sure to have correct material, e.g. correct Petri dishes for that specific media
c. Validation of media
-always include negative control
d. Always keep records-worksheet
e. Recycled tubes must be properly cleaned and autoclaved.
f. Recycled tubes that are old and scratched on the outside should be avoided, because it becomes difficult to visualize the culture.

2. **Equipment**

a. Make sure that equipment is properly maintained
b. Use the correct equipment, microscope, etc.
c. Calibrations of pipette

3. C-ELISA

a. The temperature control is crucial for c-Elisa
b. In the kit, the reconstitution of the controls in distilled water is not clearly stated or is not at the space where it is supposed to be.
c. The test should not be read before the controls are validated
d. If one of OD values of any control is aberrant, that OD should be eliminated from the mean.
e. The plate must be covered during incubation- to avoid drying the wells
f. Tips (correct tips for the particular pipette)
g. The values of the controls for each day must be checked and compared to the control values of the previous tests
h. Compare control values of different technicians and kits
i. Always use the correct filter 450nm
j. Keep a back-up lamp for the ELISA reader
k. Always keep records (worksheet)

4. CFT

a. We should all agree at the temperature for de-complementation (inactivation) 56^0C, 58^0C, 60^0C.
b. Always perform complement titration, in order to have a correct working dilution for the test.
c. The incubator must be at correct temperature 37^0C
d. Contaminated/hemolysed samples should not be used as these might give falls results
e. Correct pipetting techniques
f. The test must be validated first by checking the controls

5. PCR

a. Always include controls (pos and neg)
b. Avoid any source of contaminations
c. Preparation of samples, PCR Mix, etc must be done in separate rooms in order to avoid contaminations

d. The pellet must be thoroughly mixed in dist. Water
e. Validate the batch of reagents, each time a new batch is used (see validation protocol given)
f. Tips should be adapted to pipette (correct tips for the particular pipette)
g. Do not store PCR products, they are source of contamination
h. Do not exposure your eyes to direct UV light

6. Quality Control

a. Quality documents must be validated by a second person
b. Traceability of the sample is crucial for quality assurance (keep records)
c. For quality assurance purposes, it is advisable to purchase commercial media
d. Worksheet must be completed at every step of each test.