Conclusions
Performance of the tests

cELISA

Problems with the consistency of test results between laboratories using a different test format, suggested the use of a test kit formulation using precoated plates. The new test format was regarded as an improvement in the repeatability and reproducibility of test results. Supply of kits and availability of reagents was regarded as adequate throughout the lifespan of the programme. Assay quality control is an important factor in the general acceptance of in any diagnostic tool. The IQC data showed a good repeatability and a good reproducibility of the test. The cELISA is easy to use and regarded as highly practical under the prevailing laboratory conditions. The test appears to be robust and can be used on hemolysed or "anticomplementary" sera. The test is primarily regarded as a herd test and esting sera in single wells appears to have many advantages over testing in duplicate wells and does not seem to influence the diagnostic results provided that the IQC variations are limited. However the software used at present (EDI) does not allow an easy reading and recording of the results.

CFT

The test performs well provided that the laboratories use it on a regular basis. Problems were encountered in the availability of reagents. At present there is no single source of reagents. The repeatability and reproducibility of the CFT is affected by the differences in quality of the antigen and the combination of the reagents used. The reproducibility between laboratories has not been assessed by this program. The overall sensitivity of the CFT and the cELISA appears to be similar but the number of animals identified depends on the stage of the disease. The CFT detects antibody to MmmSC earlier in infection whereas the cELISA detects antibodies for a longer period (see results from Mali).

The specificity of a test should only be determined in a disease free area. The results for specificity from Botswana were similar to the cELISA and almost 100%

LAT

The three LATs ("Blue", "White", "Red") produced highly variable results (see table). The results appeared to be influenced by the operator although the tests were performed under laboratory conditions. There is a need for more precise SOPs. Discrepant results were observed with negative sera in Botswana (98% specificity, "Red" LAT) and Namibia (0% specificity, "White" LAT). This calls for renewed validation and interlaboratory comparisons of test performance through the exchange of reference sera and new reagents. This test was not validated under field conditions and this issue should be addressed once the preliminary laboratory validation proves promising.
RECOMMENDATIONS

**cELISA**
- As a result of the comparative studies of the CRP and the validation carried out the meeting recommends that the cELISA is used as a diagnostic tool for CBPP and be adopted by the OIE at the same level of recognition as the CFT.
- Lacking software for data expression is a major constraint and a new software is urgently needed to facilitate the analysis of test results. This software should be provided by FAO/IAEA.
- To confirm the reliability of the test results IQC data should be reported as an integral component of these results. Continued monitoring of the IQC results should be carried out to confirm the performance of the test in the laboratories where the test is used.
- An independent external quality control should be established, at the regional level, to monitor data generated by the different labs.
- The supply of reagents from a single source in a kit form was regarded as a distinct advantage and the further supply of kits should be assured from national programmes (PACE etc.).

**CFT**
- Each laboratory should establish its own standard reference serum and calibrate it against the official OIE reference sera.
- SOPs for CFT as a screening test have to be established and should be adopted by the OIE.
- IQC data should be reported for the CFT in a manner similar to the cELISA, namely by plotting the results of the reference sera and the titre of the complement.
- Reagents should be made available from a single source in a kit form to improve the consistency of results and to facilitate the comparison of CFT test results between laboratories.

**LAT**
- The "Blueii" LAT was found to be unspecific and should be further evaluated for specificity along with the "Whiteii" LAT.
- Further studies on the specificity of the "Whiteii" LAT should be carried out in Namibia (with sera from the non infected zone), Botswana and other CBPP free countries with a new batch of reagents.
- For the "Rediii" LAT similar studies should be performed for specificity in Namibia and from acute infections in Mali.
- The usefulness of the "Rediii" LAT for the detection of antigen in pleural fluids and lung fluids needs to be further evaluated. It is recommended that more precise SOPs are developed for the LAT tests to ensure uniformity of test results under field and laboratory conditions.

**i-ELISA**
- There is not enough data available to make any statement about the performance of the i-ELISA.
Surveillance and testing strategies (serological) in different disease situation

Confirmation of outbreak

The cELISA and the CFT detect antibodies to MmmSC at different stages of infection and no test can detect all infected animals and these tests, when used separately, cannot detect every infected animal with antibodies. The relative sensitivity of the two tests is between 50% and 80%. For the confirmation of an outbreak it may therefore be necessary to use both tests. In an outbreak it is recommended to collect sera from 15 animals that have been clinically affected. In the case of an outbreak of CBPP many sera will be positive and the titres will be high. The influence of antibiotic treatments on the performance of the serological tests has not been assessed and should be investigated. The isolation and identification of MmmSC was not evaluated as part of this programme, but it is essential in new outbreaks and the strains should be forwarded to the reference laboratory.

Prevalence studies in endemic areas (by serology)

Limits of the sensitivity and specificity of the CFT and cELISA to carry out prevalence studies were evaluated during the programme. The purpose of prevalence studies is to determine the proportion and distribution of infected herds. The results of both tests can be influenced during the first four months by vaccinations. Prevalence studies should be primarily based on cross sectional serological studies. Samples should be collected at least four months after the last vaccination. An initial prevalence estimate in the PACE countries could be based on the samples collected in connection with the serosurveillance of rinderpest and on the existing serumbanks. Based on the results of these estimates, CBPP specific sampling frames should be developed. Both serological tests (cELISA, CFT) might be recommended for mass screening but the cELISA can be better quality controlled and appears to be more suitable for mass screening.

Disease detection in endemic areas

Serological surveillance should be used to support abattoir and clinical surveillance. Although serology can be used to direct further clinical investigations it is not sufficient on its own to identify all herds with active disease. If serology is used it can assist to identify priorities for further clinical and outbreak investigations (see under outbreak investigations). These are as follows:

1. Herds from areas which had been classified as positive by the CFT and the cELISA and areas where clinical reports of CBPP exist.
2. Herds from areas which were identified by CFT with high titres
3. Herds from areas which were identified by cELISA with high titres

Disease detection in buffer zones

Intensive vaccination in diseased areas of buffer zones might influence the reliability of positive serological results by cELISA and CFT despite the high specificity of the two tests in disease free areas. When interpreting the serological results it is important to consider the time of the last vaccination since both tests will detect some vaccinated animals up to 4 months.
**Disease detection in surveillance zones**

Abbatoir, clinical and serological surveillance should be carried out in diseases free zones. The early detection and confirmation of disease is of highest importance. The sensitivity of serological detection should be maximized by using the CFT and the cELISA in parallel.

**Clinical surveillance**

The success of clinical surveillance relies on the ability of the veterinary services, cattle owners and other stakeholders to correctly identify suspect cases of CBPP. Considerable efforts have already been made towards the enhancement of awareness of CBPP and it is recommended that continued training in disease recognition and sample taking is provided. To facilitate disease recognition and reporting a case definition for CBPP should be developed and applied. Disease reporting of all suspect cases and laboratory confirmation is a key issue.

**Surveillance at the abattoir**

Clinical and abattoir surveillance for suspect and pathognomonic lesions can be expected to identify the majority of new outbreaks. Serological studies should be used to identify the infected herds in the surrounding areas.

Lung samples and pleural fluids should be submitted to the laboratory when CBPP is suspected. The antigen detection LAT (“Rediii” test) if it is shown to work will be of value in identifying samples to be submitted Mycoplasma isolation and where available PCR should be used to confirm the diagnosis.

**Importation in disease free zones**

Importation should be done only from other disease free zones in order to prevent the re-introduction of the disease. Even so, a quarantine has to be established before the animals are allowed to enter the disease free zone (for the surveillance of any disease and not only CBPP). Again both the CFT and the cELISA should be used on these animals. It is also recommended that such animals originate only from a herd which has been tested and shown to be clear of CBPP.

**Monitoring of vaccinations**

Monitoring of CBPP vaccination campaigns is needed to assess the effectiveness and coverage of the vaccinations.

Regarding the monitoring of vaccination coverage and the success of vaccinations no agreement was reached on the procedures.

Possible strategies are:

- Branding of vaccinated cattle by brands identifying the time of vaccination and monitoring of the proportion of cattle branded.
- Monitoring the reduction of clinical disease incidence.
- Monitoring the reduction of serological prevalence.
- Direct serological monitoring of vaccine marker (not available at present).
- Serological monitoring on MmmSC antibodies induced by vaccination (no reliable test available at present).

The monitoring should be carried out following agreed sampling frames.
Responsibilities

All diagnoses must be confirmed by the country’s Central Diagnostic Laboratory. Three laboratories actively working on CBPP namely the CVL, Bamako - Mali, to cover West and Central Africa; the NAHRC - Ethiopia, East Africa; and the CVL, Windhoek - Namibia for Southern Africa act as coordinating centres for diagnostic tests.

Appropriate samples for CBPP diagnosis (serum, lung, lymph nodes, pleural fluid) should be sent in appropriate containers under suitable transport conditions by the fastest means to the national laboratories. Samples from outbreaks might be sent to the regional laboratories for further confirmation.

Results of diagnostic tests in CBPP should be reported without delay to the Director of Veterinary Services for early response to CBPP outbreaks. All reports especially disease confirmation from reference laboratories should be reported to the OIE and neighbouring countries.

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i Blue test. Coated with whole MmmSC. Designed to detect total MmmSC antibodies
ii White test. Coated with MmmSC capsular polysaccharide. Designed to detect CPS antibodies
iii Red test. Coated with anti-MmmSC capsular polysaccharide (CPS) IgG. Designed to detect CPS (principle circulating antigen)