REPORT

of the

SESSION OF THE RESEARCH GROUP OF THE STANDING TECHNICAL COMMITTEE

of the

EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

Held at

Gerzensee, Berne, Switzerland

16-19 September 2003

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INTRODUCTION

A Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EUFMD) was held at Gerzensee, Berne, Switzerland from 16 to 19 September 2003.

The meeting was chaired by Dr Kris De Clercq (Belgium). Members of the Group present were: Drs. Aldo Dekker (the Netherlands), Chris Griot (Switzerland), Bernd Haas (Germany), Per Have (Denmark), François Moutou (France), Vilmos Pálfi (Hungary), David Paton (UK), José Sánchez-Vizcaíno (Spain), Ms Nilay Ünal (Turkey) and Hagai Yadin (Israel). Apologies were received from Dr Franco De Simone (Italy) who was unable to attend.

The acting Chairman of the Research Group, Dr Kris De Clercq, welcomed everybody to the Session and gave the floor to the Chief Veterinary Officer of Switzerland, Dr Hans Weiss. Dr Weiss welcomed the participants to Switzerland and was pleased to note that so many FMD experts could come together in this way. He thanked Dr Griot for organizing the arrangements for the meeting. As a client of laboratories he had to ask himself what he would do if FMD came to Switzerland. Emergency plans required to be tested, and will be tested, and the active contact between laboratories will be very important in peace time to ensure laboratories are better aware and able to respond to emergencies. Research networks for infectious diseases should be a platform for development of new diagnostic tests and vaccines. He wished the Session a very productive meeting.

Dr Griot then welcomed the Research Group to Gerzensee Study Centre, on behalf of the IVI and reminded the Group of the importance of our work for our clients, the veterinary practitioners and farmers, and the importance of communication of our activities and findings.

The Secretary of the EUFMD Commission then thanked the CVO of Switzerland for hosting the meeting and thanked Dr Griot and his staff for their immense efforts in the arrangement of the Session. He added that the site of healthy and contented animals at pasture in the beautiful environment reminded us all of the great progress made in Europe in the last 50 years in FMD control, and the importance of our task to maintain the high health status across our region. He brought the attention of the meeting to the conclusions and recommendations of the General Session of the Commission’s member states in April 2003. The work of the Research Group was recognized as very valuable and to be encouraged, but priorities for activities for the biennium 2003-2005 should be identified at the 2003 Research Group Session, to address the major technical issues identified at the General Session. The programme for this Session was set by the discussion of the General Session and the concern of the member states on these issues. He reminded the members that through the 1990s the Research Group had provided leadership and foresight in the technical development of tools for FMD control and the practical implication of their uptake and utilization. The Group had very much taken the international lead in the maintenance of standards in FMD diagnosis and this issue remained as important as ever. He brought the attention of the Session to the fact that in 2004 the Commission will celebrate 50 years of existence and that this biennium will be
an important time to reflect on what has been achieved and how to move forward in our field. Finally, he reminded the Session that the Chair of the Research Group is elected at the Session, and called for nominations. He thanked Dr De Clercq for his immense contribution as Chairman over the past biennium and hoped that the “mountain” of the agenda could be successfully scaled by the assembled international team.

*Presentation by Professor Vincenzo Caporale, President of the Scientific Commission on Animal Diseases of the OIE*

Professor Caporale reminded the Session that the Scientific Commission was a new title for the former FMD Commission of the OIE, and he outlined the new approach to be taken to address outstanding issues. The OIE certifies the health status of countries and zones for four diseases, FMD, CBPP, BSE and RP. The new approach is to develop ad hoc groups to deal with particular parts of the SCAD terms of reference. The ad hoc groups being developed are for Surveillance Guidelines, Regionalization, zoning and compartmentalization, country recognition (status with respect to disease), vaccine banks, and non-structural protein (NSP) antibody tests for FMD serology. He indicated that surveillance guidelines were required for each of the four diseases where country status is certified, as well as generic surveillance guidelines for terrestrial animal diseases. The surveillance guidelines for FMD need to cover the surveillance standards in the demonstration of freedom from infection in a population regularly vaccinated, in an emergency vaccinated population, and in a non-vaccinated population. The performance of tests at individual and population level was important to determine, for all relevant species. Measures to ensure product safety was also an issue, particularly to identify safe trading practices and commodities for countries that can be applied regardless of FMD status. The safe trade of meat from vaccinated pigs was one situation to be resolved. He suggested that the EUFMD Research Group could play a valuable role in supporting the work of the SCAD ad hoc groups, and that it would be advantageous for the Research Group to work with experts in other regions, such as South America, to develop joint technical papers and opinions.

**Adoption of the Agenda**

The Chairman proposed that the following Agenda should be adopted:

- **Item 1** Briefing on relevant research projects
- **Item 2** Post-vaccinal surveillance – issues, experience, outlook and post-vaccinal surveillance – tests for differentiating infected and vaccinated animals (DIVA)
- **Item 3** Priority setting for FMD vaccine bank (risk assessment/true prevalence of FMD)
- **Item 4** Towards virus detection standards – including RT-PCR
- **Item 5** Diagnostic standards – Reference sera
- **Item 6** “Rapid diagnostics” for FMD
- **Item 7** Contingency planning for FMD laboratories
- **Item 8** Guidelines for air transportation of FMD samples
- **Item 9** Critical review of inactivation standards
- **Item 10** Work programme 2003-2005
- **Item 11** Any other business
Item 12 Open papers on emerging issues/latest developments
Item 13 Presentation and adoption of report

The Agenda was adopted as proposed.

**Item 1 – Briefing on relevant research projects and applications supporting FMD research in Europe**

Dr Kris De Clercq briefed the session (Appendix 1) on proposals developed and submitted for EC funding in the last year, that had the intention to strengthen the research effort and the network of FMD expertise in Europe. These were:

1. “FMD improCon”, a specific research project oriented to support EU policies, that was successful in the first stage of evaluation and is presently under contract negotiations.
2. Reference standards development, that is under consideration by DG-SANCO.
3. European research area network (ERANET) application submitted in June 2003 and which after evaluation may be resubmitted when the eligibility of partners is established, and
4. Project for community and national reference laboratories to strengthen collaboration, which remains to be written in late 2003, with the coordination of the WRL and which should involve FMD and CSF laboratories.

He identified gaps in the European programme to be:

1. Evaluation and other studies relating to contingency planning.
2. Work to develop reference virus isolates/genomes.
3. FMDV survival in animal products.
4. Virus transmission studies, through direct and indirect contact.
5. FMDV vaccine evaluation.

**Discussion**

It was strongly suggested that socio-economic aspects that relate to infectious disease control should not be neglected. Further, operational research methods could be very important in the evaluation of contingency plans. The new EU directive, which should shortly be in force throughout the EU, should provide a legal basis for support to resolve some of the technical issues which constrain policy application. The Session greatly appreciated the effort of Dr De Clercq to identify funding options and to coordinate proposals which address the key areas of concern to the Research Group.

**Item 2 – Post-vaccinal surveillance (PVS)**

*I - Issues, experience, outlook*

The Secretary of the Commission outlined a number of recent developments relating to surveillance after the use of vaccination in previously free, non-vaccinating countries.
These include the Guidelines for FMD surveillance prepared by the FMD Commission of the OIE, which have been accepted by the International Committee of the OIE in May 2003 but which will be further reviewed and are expected to be revised before May 2004. A difficulty in developing FMD Guidelines has been the lack of definition of an acceptable level of evidence for absence of virus infection in a vaccinated population. He suggested that collaboration with countries such as Uruguay which had conducted significant post-vaccination surveillance could be instructive to the better definition of guidelines and had for this reason invited Dr Andrés Gil to the Session. Further, he indicated that there is some flexibility allowed in the OIE Guidelines that could be beneficial to countries to enable the selection of surveillance strategies are most cost-effective for their epidemiological situation, and where the sampling strategy may be adjusted to compensate for test performances. He indicated that confidence in the surveillance design and sampling to detect previously infected animals was very important, and that quantitative methods to demonstrate confidence in the absence of infection could be very valuable, and for this reason had invited Dr Matthias Greiner to the meeting. Under the articles of the new Directive, tests would be used to classify herds and therefore confidence in the selection of test systems, and the criteria for positive results, will be very important. Issues relating to tests selection need to be resolved, and new methods for test interpretation that could add confidence to the detection of carrier or previously infected animals, should be evaluated. For this reason the potential use of likelihood ratios would be explored at this meeting.

Dr Andrés Gil presented a paper (Appendix 2) on the sero-monitoring for FMD infection following the 2001 type A epidemic in Uruguay.

Dr Nilay Ünal presented the preliminary findings (Appendix 3) of sero-surveillance in Anatolia and Thrace regions of Turkey following the 2003 spring vaccination campaigns.

The importance of PVS is linked to the fact that emergency vaccination around outbreaks could be realized in Europe as a response to FMD outbreaks. Rapid recovery of FMD free status without vaccination will be essential for economical reasons.

Questions are linked to the possibility of virus animal carriers, but also to the definition of notions like “active virus circulation” and “infection freedom”. In this context, little in the way of data on within herd and between herd infection rates, in relation to time-space and to the time-course of outbreaks and vaccination measures, are already available and so, those existing should be very important to analyze.

Dr Matthias Greiner presented a paper (Appendix 4) on novel approaches to the use of surveillance data to demonstrate confidence in the demonstration of freedom from infectious diseases, using the example of CSF surveillance in Denmark. The approach was developed to identify and quantify the role played by “routine surveillance” operations, such as farm visits, abattoir inspections, in the surveillance for CSF, and thereby to reduce the need or cost of structured sampling/sero-surveillance activities. The methodology will be presented during a post ISVEE workshop supported by the OIE and the international EpiLab, in November 2003, in Vina del Mar, Chile. The surveillance activity maybe targeting in different ways populations with higher risks and populations with lower risks of infection, and could be of considerable value to the demonstration of
FMD freedom. Over sampling in the higher risk population must not compromise the probability to sample adequately the lower risk population. A targeted surveillance activity may enhance probability of finding the disease and then reduce the costs of surveillance. This must be relevant also for PVS, with the importance of NSP serology, and of clinical and slaughterhouse surveillance in non-vaccinated sentinel animals.

**Recommendations**

It is recommended that EUFMD secretariat could approach Uruguay authorities to have the possibility to work with them on their data. A precise knowledge of within herd prevalence and of between herds prevalence from the large serological surveillance surveys realized in this country could be very useful to address the PVS issue, even if the context may be different from the European situation. The whole surveillance system is important, but the serological data are specifically relevant.

In the same way, the serological surveys realized in Turkey could also bring very useful information on this issue. The low percentage of positive results (less than 2 per cent) with the 3ABC test could also be seen as a specificity question, not only as a possibility of a low level of circulation of the virus. Further investigation of the results and the circumstances of the villages which had positive animals is recommended.

The unsatisfactory level of antibodies to structural proteins post vaccination should be investigated by further data analysis.

Methods to quantify confidence in the absence of disease or infection that use non-serological and serological data should be explored for the context of demonstration of FMD freedom in non-vaccination/vaccination scenarios.

**II - PVS tests for differentiating infected and vaccinated animals (DIVA)**

Two papers on the evaluation of tests for use in PVS were presented, by Dr David Paton (Appendix 5) and Dr Aldo Dekker (Appendix 6). One paper was presented by Dr Michael Collins (Appendix 7) on the use of likelihoods ratios to assist in the interpretation and communication of ELISA data.

Experiments at Pirbright involving contact challenge of vaccinated cattle demonstrated complete clinical protection, but a variable degree of protection against viral replication, with animals either 1) being completely protected from virus replication, 2) supporting transient virus replication, or 3) becoming carriers. No single test detected all of the carriers. Commercially available NSP test kits were not equally effective at detecting infection in vaccinated animals. An in-house test for specific IgA in saliva was the most sensitive approach.

The issue of correct diagnosis in serological tests, and methods to express the probability of correct test result, was explored by Dr Collins. He presented a pilot study of the use of likelihoods ratios (LRs) of a correct result, in the detection of FMD virus challenged animals, using data supplied by the IZS, Brescia. LRs can be expressed at a range of cut-off values, and therefore for each test result, provide an indication of the likelihood of this
result occurring by chance. For this reason, assurance in the likelihood of a correct diagnosis can assist in difficult decisions, such as slaughter of herds containing test positives. The observation that high ELISA values were obtained in most virus challenged, vaccinated animals, with two NSP tests (Bommeli and an in-house assay) and also in tests for antibodies to SPs (the rise in antibodies on challenge) resulted in potentially useful LRs with each of the NSP test, and the use of SP tests. A LR of >100 was observed using the Breschia in-house NSP test for results >40% of the positive control. However, he warned that use of the method would depend on the validity of the experimental data in relation to field exposure of vaccinates, and the results should be considered preliminary findings.

Dr Dekker presented a comparison of 5 DIVA screening/screening plus confirmation tests on a selection of sera from cattle of known status. The ELISAs were obtained from their producer. The results were used to produce ROC curves, and compare the tests using a permutation test. The curve of the Ceditest was almost optimal, and significantly different in ROC curve than those obtained with 4 other tests, and a higher analytical sensitivity for detection of dilutions of the positive serum. For duration of a positive response, the Ceditest and the Aftosa test (from PAHO) were able to detect 16/16 animals at 441 days post-infection, but the specificity of the latter appeared poor. However, with vaccinated calves given intra-nasal virus challenge, the sensitivity of detection was much lower. The infection status (acute infection or carrier status) of these calves was not reported.

**Discussion**

The possibility to vaccinate herds in emergency and to recover rapidly a FMD free status is linked to serological tests able to differentiate infected from vaccinated animals. The validation of the tests may not require the testing of 300 different animal sera from all FMDV serotypes, and the recovery of FMD free status may not require only serology against NSP. The lowest acceptable prevalence and its accepted confidence value will orientate towards the most appropriate sample size. Possibilities for systematic review and systematic summary (e.g. meta-analysis) of existing evidence should be explored.

**Recommendations**

It is recommended that the definition of the number of animals required in test validation studies be approached after definition of what would be required in surveillance strategy. To do this, a thorough review should occur of the within-herd prevalence data to be expected.

It is also recommended that comparison of DIVA tests be conducted on the most significant category of animals, those which have been vaccinated, challenged by (preferably) infected animal contact, and shown to have become infected.

Close co-operation between European laboratories and those in other parts of the world is required to achieve this in the shortest time.
SATs genotypes have been less explored than other genotypes in the context of PVS-DIVA. It is recommended that evidence of a problem in detection of SAT infections after vaccination be reviewed before a recommendation on performance of animal studies be made.

It is recommended that a synthesis (meta-analysis) of all the tests be performed so that overall performance, and lack of data, if any, may be realized and completed.

Other ways to interpret test results based on use of different cut-offs and probability calculations should be encouraged as a possible means of identifying infection at herd or individual level.

**Item 3 - Priority setting for FMD vaccine bank (risk assessment/true prevalence of FMD)**

Dr David Paton presented an overview of FMDV genotype information available to the WRL for 2002-2003, and relevant antigenic characterisation (Appendix 8). Recent trends or events of importance were highlighted. Disparity in continental and regional use of the WRL services was a long term problem leading to relative lack of information from some regions, particularly in Africa and South America. Availability of reference sera for antigenic typing remained an important constraint.

Dr Keith Sumption presented the results of a survey (Appendix 9) of expert opinion on gaps in the global surveillance for circulating FMD virus, and some considerations on the use of livestock population and husbandry systems information to target surveillance. Predictive FMD maps might also assist the targeting of control efforts, including better identification of the need for vaccine antigens.

Dr Mark Thurmond presented in outline a new initiative (Appendix 10) to map FMD risk, using observed FMD data from three countries to develop models for FMD incidence and prevalence that might be adapted to address global information needs.

Dr Marius Gilbert presented an analysis of FMD types O, A and Asia-1 occurrence in time and space in Turkey (Appendix 11). Different spatial-temporal trends for the three types were observed, which may permit prediction of future FMD.

**Priority antigens for 2003**

It is recognised that we lack information regarding the characteristics and prevalence of FMD types and subtypes in some parts of the world. Furthermore, our systems for matching field isolates to vaccine strains are imperfect. Therefore, a rather conservative approach has to be taken with respect to concluding that certain vaccine strains may no longer be required. Likewise, newly emerged strains may provide locally useful vaccines, but such strains are not necessarily suited to incorporation into international vaccine banks, unless they can be shown to confer a broad coverage of protection.

Data from South America suggest that type A viruses from 2000 and 2001 show a relatively poor match to A24 Cruzeiro and that vaccines based on A Argentina 2001
should also be available. Data from the Middle East suggest that the A22 Iraq vaccine is less useful than previously and that a vaccine based on A Iran 87 would be useful. This could replace the recommendation for inclusion of the related A Saudi Arabia 23/86 vaccine. The Iran 87 vaccine may also provide cover against type A viruses from South East Asia, where the A15 Bangkok vaccine seems less useful. Given the lack of evidence for circulating type C FMD virus, it may be less important to maintain large reserves of the C Noville vaccine strain than previously.

**Priority locations from which assisted delivery of isolates to WRL is required**

A ranking of priority locations was obtained by analysis of the answers provided by experts to a questionnaire on this subject. The order of priorities obtained was:
1. China
2. Indian subcontinent
3. African horn
4. Africa East

Few or no samples have been submitted to the FMD World Reference Laboratory from these regions in the last three years.

It is instructive to look at which FMD infected countries have the highest populations of susceptible species and are the main exporters of live animals and meat, since these are likely to represent a particular threat. China and India do indeed have some of the largest populations of susceptible species in the world. Countries in sub-Saharan Africa also merit more attention than they have previously been given. A number of projects are now underway to examine in detail the ecosystems in selected countries where FMD is endemic. These studies will seek to assemble information on the location and chronology of FMD outbreaks and on the host species and FMDV serotypes and subtypes involved. This will be analysed in relation to a variety of factors that may contribute to the persistence and spread of the FMD virus such as animal density, husbandry and trading practices.

Principal constraints to sample submission are related either to concerns over the use of the submitted samples and information derived there from or to the cost and effort required relative to the perceived benefit obtained. It was concluded that in the first instance efforts should be made to encourage the submission of more FMD sample materials from Africa, since obtaining information from this region is a relatively high priority and there is a reasonable prospect of improving submissions if resource is targeted here.

**Recommendations regarding improved priority setting for vaccine banks**

1. Available information on the diversity of circulating FMD viruses and vaccine matching data should be pooled by improving the liaison and exchange of information between regional reference laboratories. A project in the framework of the EU ERA Net will be submitted in the spring of 2004 to take this objective forward. A useful initiative would be to organise meetings of representatives from regional reference laboratories. These could take place every two years. The
World Reference Laboratory will examine the costs and feasibility of organising a first meeting in early 2004.

2. The value of vaccine matching tests on available field isolates should be improved by procuring a more representative supply of vaccine strains and vaccine antisera. This requires closer liaison between vaccine companies and Reference Laboratories. Consideration should also be given to funding reference laboratories to produce their own supplies of reference antisera by Reference Laboratories.

3. Research is needed to improve, standardise and validate in vitro vaccine matching tests. This includes in vivo cross-protection studies and more work to characterise the antigenic sites critical to protection. A new research project under the EU 6th Framework is close to agreement and will support these objectives.

Recommendations regarding improving the estimation of FMD and antigenic type prevalences

1. The submission of more FMDV samples to the World Reference Laboratory should be encouraged to enable genetic and antigenic characterisation studies to be performed. Better liaison with Regional Reference Laboratories may encourage the supply of representative viruses from their collections to the World Reference Laboratory (see recommendation 1 above). Efforts to encourage and subsidise submissions from endemic countries should concentrate initially on targeting resources to countries in sub-Saharan Africa and the horn of Africa where it is likely that financial assistance could have the greatest benefit. The EUFMD Secretariat should co-ordinate such an approach.

2. New research to improve the knowledge of the ecosystems in which FMD is endemic has great potential for identifying the mechanisms by which the virus persists and spreads and thereby to develop risk assessments and new control strategies. The groups should be invited to update the EUFMD on progress in their research in the coming years. The EUFMD Secretariat and the FMD World Reference Laboratory should support these initiatives and help to co-ordinate the activities of different research groups. Discussions and agreement are needed on the extent to which surveillance data received from National Governments by organisations such as international reference laboratories can be made more widely accessible.

Recommendations from the World Reference Laboratory on FMD virus strains to be included in FMDV antigen banks (2003)\(^1\)

The virus strains listed may have equivalents that could be considered as alternatives.

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\(^1\) Provisional recommendations at the Session were modified by WRL after further review; O Campos replacing O Lausanne; A Iran 87 moved down to medium; A Saudi 23/86 added as medium priority; A Eritrea 98 and A 87 Argentina moved down to low priority.
High Priority

O Manisa (*covers panasian topotype*)
O BFS or Campos

A24 Cruzeiro
Asia 1 Shamir
A Iran ‘96
SAT 2 Saudi Arabia

(not in order of importance)

Medium Priority

SAT 2 Zimbabwe
A22 Iraq

SAT 1 South Africa
A Malaysia 97
A Iran 87¹ or A Saudi 23/86¹
A Argentina 2001
O Taiwan 97 (*pig-adapted strain or Philippine equivalent*)
A Iran ‘99

(not in order of importance)

Low Priority

C Noville
A15 Bangkok related strain
A Eritrea 98¹
A 87 Argentina related strain¹
SAT 2 Kenya
SAT 1 Kenya
SAT 3 Zimbabwe
A Kenya

(not in order of importance)

**Item 4 - Towards virus detection standards – including RT-PCR**

A paper (Appendix 12) provided by Dr. Wolfgang Philipp and Dr. Heinz Schimmel of IRMM was presented by Dónal Sammin, outlined the requirements of reference system and the steps required in its establishment. For every reference system, both a reference method and reference material are required. A reference material should be commutable (i.e. representative of the normal test analyte) and should be homogenous and stable, giving reproducible results. A reference method should be validated and follow an SOP. Within laboratory variation should be evaluated and all steps should be quality assured.
In the framework of FMD diagnosis, OIE prescribed methods can be taken as reference methods.

Dr. Anton van Loon gave a presentation on the work carried out by QCMD (Appendix 13), formerly the concerted action EU-QCCA. Both the number of programmes which were conducted and the number of participants involved was very impressive. They have shown that it is possible to organize complex proficiency trials and that these can improve laboratory efficiency in diagnosis. They have demonstrated a large difference in analytical sensitivity between laboratories, even when using the same commercially available method. Based on their experience future proficiency panels will use only those dilutions and strains that are clinically relevant as the basis for scoring laboratory performance, although further dilution series and unusual strains will still be included for scientific interest. Dr. van Loon recommended that two PCR processes are performed on each sample; one for agent of interest and the other to evaluate PCR inhibition.

Dr. Kris de Clercq presented a paper (Appendix 14) on current status and deficiencies with regard to FMD virus detection. For FMD virus detection, regardless of the method employed, there are no reference materials available and none of the available methods are suitable for large throughput with high sensitivity, although progress is being made with automated RT-PCR. The current threat of SAT-2 emphasizes the need for diagnostic capability for all seven serotypes.

Conclusion

Reference material is essential in the framework of a QA/QC system and should be produced for virological and serological FMD diagnosis.

Recommendations

1. To establish a proficiency panel for virological testing according to the following timetable:
   - Set up a subgroup to decide on a protocol for preparing a proficiency panel for VI, RT-PCR and ELISA (before January 2004).
   - Distribute proficiency panel for VI, RT-PCR and ELISA to limited group of laboratories (before June 2004).
   - Report the results of testing the proficiency panel for VI, RT-PCR and ELISA (before September 2004).
   - Adopt a protocol for conducting a proficiency test for VI, RT-PCR and ELISA (at the RG session in 2004).
   - Distribute proficiency panel for VI, RT-PCR and ELISA to all national reference laboratories (before June 2005).
   - Report the results of testing the proficiency panel for VI, RT-PCR and ELISA (before September 2005).

2. That the long-term objective should be the development of Reference standards for VI, RT-PCR and ELISA (before 2007).
**Item 5 - Diagnostic standards – Reference sera**

Dr. David Paton reported on the results of Phase XVII and presented a plan for completion of Phase XVIII (Appendix 15). There is a difference between selection of reference sera and proficiency testing. OIE guidelines state that a strong positive serum, weak positive serum and negative serum are required. Of these, the weak positive serum is the most important. The aims for phase XVIII are:

- Introduction of SPCE
- Preparation and reporting results from secondary standards
- Use of calibrated tests to examine local negative serum panels
- Use of calibrated tests to examine proficiency test panel
- Standardization of internal quality control procedures
- Possibly evaluate new Reference Sera with NSP ELISAs

These aims were approved by the Session.

The session discussed whether reference sera for the SAT-2 serotype should be produced as a matter of urgency for calibration of serological tests for the SAT-2 serotype in the member countries, because of the threat of introduction from Libya, Dr. Have reminded the session that NSP tests can be used for this purpose, as they are not serotype specific. The Group recommended that work continue with the introduction of the SPCE, and encouraged those undertaking potency tests to use the SPCE in parallel to the LPBE to identify levels of response which equate with protection.

Work in progress on the validation of the solid-phase competitive ELISA for FMDV types A, C and Asia 1 was mentioned by Dr Paton and progress in stabilization of the antigen has been made to assist kit development. Further information was subsequently provided on this (Appendix 24).

**Conclusion**

There is a difference between selection of reference sera and proficiency testing and therefore the two processes should be addressed separately.

The meeting endorsed all objectives of Phase XVIII.

**Recommendations**

1. The timetable for the completion of Phase XVIII should be:

   - Material for testing in the process of Phase XVIII should be distributed before December 2003.
   - National Reference Laboratories in countries which do not use vaccination should test sera from at least 1000 non-vaccinated non-infected cattle preferably in the calibrated SPC ELISA. This should be reported to the WRL together with the quality control data, including results on the secondary standards, and the results of the proficiency panel before May 2004.
The report on the results of Phase XVIII should be distributed before September 2004 and discussed at the 2004 RG Session.

**Item 6 - Rapid diagnostics for FMD**

Prof. José Sánchez-Vizcaíno presented a paper (Appendix 16) on a multiplex RT-PCR combined with restriction enzyme analysis for FMDV, SVDV and VSV detection. For each virus type several isolates were tested with a positive result. A significant result was the fact that the SVDV primers did not detect Coxsackie B5 virus.

Dr. Malik Merza showed information on the development of pen-side test for Rinderpest and FMDV (paper requested but not supplied by author). Validation of the tests is ongoing. Svanova also developed a 3ABC ELISA using a recombinant 3ABC protein from Argentina. The protein was modified in the 3C region to increase stability. Dr. Merza showed initial validation data, which gave promising results. Further validation is necessary, he invited everyone to send positive sera from vaccinated and infected animals from different parts of the world. He mentioned the use of an oligonucleotide ligation assay also known as “padlock” technology as a signal amplification system. In interleukin assays this technique was far more sensitive than conventional technique.

From the discussion on the use of pen-side test two major issues arose. Firstly, the session discussed the performance of pen-side tests compared to laboratory based tests. Secondly, the application and interpretation of pen-side tests were discussed. By whom will the test be performed, and for what purpose will the test be used.

Pen-side tests could be used to:

1. Support early detection of infection in suspect clinical cases (primary/index case).
2. Confirm clinical diagnoses in secondary cases.
3. Rapidly indicate the necessity for additional sampling.
4. Confirm the clinical diagnosis of FMD in the absence of a laboratory infrastructure.

Pen-side tests should only be used by official veterinarian in the course of a disease investigation.

**Conclusion**

Pen-side tests should be rigorously validated and guidelines should be developed for their application and interpretation.

**Recommendations**

1. The use of pen-side tests for FMD antigen detection should be encouraged to support the validation of the test with field data.
Item 7 - Contingency planning for FMD laboratories

Dr Dónal Sammin presented the results of a questionnaire survey on the sero-diagnostic capacity of FMD laboratories in EUFMD member countries (Appendix 17). The number of animals tested increased almost 50 fold between 2000 and 2001. With the exception of 4 countries, which did not respond to the questionnaire, all member countries either have a FMD reference laboratory (20 countries) or rely on the service of another member country (7 countries). The monthly testing capacity of member countries as percentage of susceptible animals varies, but with 3 exceptions is less than 1 % and in 8 countries is below 0,1%. In a supplementary questionnaire, information on staff, involvement in contingency planning, types of serological tests performed (mostly LPBE), reagent stocks, production and supply was asked. It was found that existing stocks would often be used up before they could be replaced.

Dr Bernd Haas presented a paper (Appendix 18) on a potential diagnostic test kit / reagent bank for Europe. New control strategies lead to an increased demand for laboratory investigations, especially serological screening. In “peace times” only a limited number of samples is tested, mostly using in-house tests of the national reference laboratories. However, after an outbreak of FMD, laboratories will be expected to quickly scale up their serological screening capacity to levels reaching or exceeding the full capacity of all the countries veterinary laboratories combined. This is a problem especially for countries with a decentralized laboratory structure, where regional laboratories that have no experience with FMD serology would have to take over most of the testing. This will require long lead-in times unless they are supplied with complete, commercially produced test kits. Even if mass serology and FMD diagnosis is performed by the same central laboratory, lack of reagents and test kits could slow down the implementation of disease control measures as well as the lifting of restrictions. However, with a proposed bank, the lead-in time until tested reagents and, preferably, complete tests kits, are delivered in sufficient numbers, could be shortened significantly. The capacity of such a bank should be sufficient to allow a major country to make full use of its testing capacity during the first weeks after an outbreak. Pre-vaccination blood testing carried out in the Netherlands in 2001, as previously shown to be of value in investigation, will require a shorter lead-in time.

Dr François Moutou gave a talk on modelling of FMD epidemic size (Appendix 19). He stated that estimating the size of epidemics is very difficult, not only because of insufficient data, but also because each past outbreak has a very low probability to be seen again and no “average” outbreak exists. The earlier in an outbreak modelling of the epidemic is attempted, the less precise the results will be - whereas the benefits of good predictions would be greatest in the beginning. Important factors to predict the size of an epidemic at an early stage would be the agricultural structure of the affected area, trade pattern, animal density, species involved, age of lesions and the number of apparently “primary” outbreaks.
Conclusions

Serodiagnostic capacity has increased sevenfold since 1995. However 81% of the capacity is concentrated in four countries and three countries reported a monthly capacity equivalent to 1% or greater of the susceptible animal population.

Because of the difficulty to predict the size of outbreaks, modelling is of limited use for diagnostic contingency planning, which should rather try to shorten the lead-in period needed until the full capacity of the countries diagnostic laboratory system can be employed for FMD serology.

Diagnostic test kit / reagent banks are considered an essential part of contingency planning. Without them, the availability of suitable tests could become a limiting factor for the implementation of disease control measures and for the rapid lifting of restrictions once the outbreak appears to be under control.

Validation of NSP tests has now reached a stage, that they could be used for the detection of infection in vaccinated cattle populations as well for the screening of unvaccinated cattle and pig populations (e.g. the situation where there is a lack of suitable type specific testing capacity).

Producers should be involved in the consultation phase before drafting of a plan for a diagnostic test kit / reagent bank in order to define a structure that provides optimum performance for the available budget and avoids the necessity to discard components because their shelf live has expired.

Based on experience and theoretical considerations, it is considered justifiable in emergency situations to carry out serological tests in regional laboratories that do not fully meet the standards for FMD laboratories. However, this applies only to samples from holdings without any clinical signs of FMD. Furthermore, these regional laboratories would need to implement additional biosecurity measures.

Recommendations

Contingency plans for serodiagnosis should prepare the veterinary services and laboratories for large scale serological screening, including identification of the likely lead-in time for such testing.

An European diagnostic test kit / reagent bank should be established in order to ensure the rapid availability of suitable complete test kits, or in cases where such kits are not available, tested reagents in sufficient quantities. This bank should contain test kits for antibodies to NSP suitable for testing of cattle and pig populations. However, serotype-specific test kits / reagents for “anti-structural” antibodies should also be included, because they will still be needed for certain purposes, e.g. testing of small ruminants.
A working group should be established involving EUFMD research group, WRL, SANCO to prepare a recommendation on the structure of a European test kit / reagent bank.

In order to support decisions on which tests should be bought in which quantities for the European diagnostic test kit / reagent bank, a data base should be created and managed by the WRL. This data base should contain the results of validation studies performed with test kits that are ready to be marketed, as well as data on laboratory capacities and epidemiological data which could be expected to support this decision.

The security standards for FMD laboratories\(^2\) should be reviewed. An amendment should be included for laboratories performing only serology on samples from holdings without clinical signs of FMD.

**Item 8 – Guidelines for air transportation of FMD samples**

Dr Vilmos Pálfi gave an overview (Appendix 20) about the regulations for the transport of infectious dangerous goods by air. He presented information on the most important publications and websites on this matter and emphasized the importance of communication and coordination between shipper and consignee for the safe and reliable arrival of the samples in good conditions. The responsibilities of the shipper, the consignee and the carrier were explained. The lack of knowledge and funds (e.g. for licensed packaging materials) were found to be the most important reasons for problems in sending materials to the WRL.

**Conclusions**

Selection, collection, packaging and shipping of specimen submitted to the WRL for the diagnosis of vesicular diseases require a very specific knowledge.

Shippers of infectious dangerous goods need proper training in the packaging of materials and the preparation of shipping documents according to the current IATA and ICAO regulations.

The WRL recommends that samples are sent as airfreight and not by a door-to-door courier service in the transportation.

The relevant information on these topics is available, but has to be compiled from a number of separate sources.

**Recommendations**

A manual containing the principles of selection, collection, packaging and shipping of specimen to the WRL should be compiled. It should include the specific IATA and IAH rules for sending vesicular material to the WRL, including examples of correctly filled out documents and also links to the sources of further information. It should be part of the

\(^2\) Published as an appendix in the Report of the Thirtieth Session of the EUFMD, held in April 1993. Full document will be available in the EUFMD Website.
website of the WRL. EU member states that are required to use couriers should ensure the samples arrive at the authorised agent of the WRL.

The EUFMD Secretariat should alert the member countries to the importance of supporting transportation of samples to the WRL/RRL from countries where FMDV surveillance is currently very limited.

**Item 9 – Critical review of inactivation standards**

Dr Per Have presented a review (Appendix 21) of methods for describing the effect of temperature and time upon virus survival in products. He summarized the risk reduction measures specified in the new Directive of the EU, under the three phases of an emergency vaccination programme. He highlighted that heat treatments which do not specify a period for heating and cooling cannot be directly compared. The current requirements for heat treatment of meat can be considered to provide a high degree of safety, when applied to low level contaminated products such as meat from vaccinated animals.

Dr Aldo Dekker reviewed the risk analysis process (Appendix 22) which could be applicable to the issue of management of the risk relating to meat and milk from vaccinated herds/animals which test negative by NSP tests, in an emergency vaccination zone. The question of acceptable was considered, since heat treatment of pork or milk can render the products of less value and marketability.

**Conclusions**

1. Animal products prepared during the waiting period from an emergency vaccination zone before regaining freedom for FMD may constitute a risk of transmitting FMD, depending on species, vaccination status, post-vaccination surveillance and product type, among others. The risk may be linked either to the presence of carriers or recently infected naive animals at risk.

2. The risk of animal products is related to the maximal amount of virus that can be encountered in that particular product. Fresh pork or fresh meat excluding offals from ruminants (carriers or non-carriers) derived from immune, vaccinated animals does not contain infectious virus, whereas fresh meat from recently infected (clinically or subclinically) animals may contain significant amounts of virus.

3. In ruminants, maturation to a controlled pH below 6, deboning and removal of visible lymphatic tissue greatly reduces any viral infectivity. In contrast, not all parts of pig carcasses reach pH below 6, hence thermal treatment is normally applied as a risk reducing step rather than maturation and deboning.

4. Thermal inactivation is widely used to reduce or eliminate microorganisms (bacteria, viruses) in animal products. The kinetics of inactivation can normally be describes as first-order, however, heterogenous populations of varying thermal resistance may exist. The effect of heating is always determined by a combination of time and temperature.
5. The decimal reduction time $D$ and $Z$-value (heat resistance) of FMDV can be derived from kinetic studies of inactivation rates in relevant products. Such studies are until now lacking or at best incomplete.

**Recommendations**

1. Commodities (milk, fresh meat and meat products...) that constitute a risk for spreading FMDV should be identified by methods of qualitative risk assessment and a priority list should be established (risk profiling and risk ranking). Specific studies on heat inactivation should be designed to support further risk assessments for those identified as “high” risk commodities. These studies should make use of existing experimental data on $D$ and $Z$-values or involve further experiments to fill any gaps.

2. The available data on inactivation of FMDV in milk and milk products should be reviewed in the light of current international trade standards. If necessary, additional studies on inactivation by heat treatment or lowering pH should be carried out.

3. Until estimates of $D$ and $Z$ have been established, a heat treatment corresponding to 70°C for 1h throughout the product can be used as an interim guideline.

**Item 10 - ACTION PLAN 2003-2005**

Progress reports on each of these items will be required at the Closed meeting of the Research group in 2004, and also where indicated below. Underlined person is designated as leader, alternate in italics.

- Assisted delivery for samples from third countries  
  Action: EUFMD secretariat (report, each Executive Committee Session)

- Vaccine selection: invite comments from vaccine manufacturers and organise workshop (Jan-Feb 2004) for regional reference laboratories, etc.  
  David Paton/ EUFMD secretariat

  François Moutou/Aldo Dekker/ Alf Füssel/Matthias Greiner/Andrés Gil

- Laboratory sero-diagnostic capacity – guidelines  (by April 2004)  
  EUFMD secretariat

- Phase XVIII  WRL  → report to RG session 2004 & plan for next phase  
  c/o David Paton (outline plan of John Anderson, WRL)
• Comparative evaluation of candidate DIVA tests, 1) with sera from experimental infections, with Panaftosa 3ABC ELISA/EITB, deadline 3 months after receipt of kits, and 2) field use in regions with FMD outbreaks, deadline August 2004
  Franco de Simone (E Brocchi)/Aldo Dekker/Bernd Haas/ David Paton
  Nilay Unal /Hagai Yadin (field use in vaccinates +/- clinical FMD; spring)
  
• Proficiency panel for virus detection methods (VI, antigen ELISA, RT-PCR)
  → Step 1: limited number of NRLs → report to RG session 2004
  → Review/plan Step 2 = distribution to all NRLs → report to RG session 2005
  c/o David Paton (& staff) + Aldo Dekker/Bernd Haas/Chris Griot/Kris deClerq
  
• Global FMD surveillance map/models
  Plan: by end of December, 2003
  EUFMD/WRL/FAO/OIE Working group
  
• Evaluate pen-side tests and develop guidelines
  Plan: by end of December, 2003
  Nilay Unal/ Hagai Yadin/EUFMD secretariat (pilot study on disease outbreak investigation)
  
  Per Have/José Sanchez-Vizcaíno/Alf Füssel/etc.
  
• Working group on development of a diagnostic reagent bank (by Cordoba, April 2004)
  Bernd Haas/Alf Füssel/Kris de Clercq etc.
  
• Guidelines for sample transport (by Cordoba, April 2004)
  Vilmos Palfi/David Paton/Chris Griot
  
• WORKSHOP on contingency planning for NRLs (April 2004; Cordoba, Spain) with local organization by José Sanchez-Vizcaíno and attendance by all NRLs. Position papers must be prepared in advance by all working groups.
  
• Study to assess D-values and Z-values for heat treatment of milk and pork from FMD-infected animals.
  Per Have to draft outline of project (by Jan. 2004) with contribution from Hagai Yadin.
  
• In 2005 RG group to review vaccine antigens and gaps in sample submissions to reference laboratories (i.e. priority antigens and locations; two-year review).
Item 11 – Any other business

1. *Election of Chairman of the Research Group*

   The Secretary indicated that no members had come forward or been nominated for the position of Chairman. He called for nominations from the floor. Dr Haas nominated Dr De Clercq and there were no other nominations. The Session indicated its strong and unanimous support for the continued chairmanship of Dr De Clercq.

2. *Future procedure for the election of Research Group members and Chairperson*

   The Secretary presented a proposal for the election procedure. He indicated that the report of this session would be presented to the Executive Committee in October 2003 and that any new procedures, if adopted, would not come into effect until the General Session of 2005. He proposed that the elected membership would normally remain at 12 persons, and that the representative of the WRL would become an *ex officio* member and thereby invited to each Session. The procedure for election of members was proposed to be through nomination by the Executive Committee of the Commission, of 6 members, with 6 members to be elected on an individual basis. The group of 6 would be voted in as a group at the General Session. The other 6 members would be elected by the General Session and terms of membership would continue to be of 2 years. The proposed terms of membership were discussed and the number of terms that could be served on a continuous basis clarified. Individual members could serve a maximum of 2 terms of 2 years, but there would be no fixed maximum for members nominated by the Executive Committee. The Chairperson of the Group would be elected from any of the members elected by the General Session, and could serve a maximum of 3 terms of 2 years on a continuous basis. Membership of the Group was discussed and was proposed to be restricted to those with internationally recognized expertise of relevance to FMD epidemiology, surveillance and control, particularly in surveillance risk analysis, virus diagnosis, vaccinology and evaluation of control options, where their expertise in the FMD field is applied on a regular basis. The group considered that alternatives should also be considered as well as the above, such as the proposal that the group to be elected following the nomination of the Executive Committee be more than 6.

3. *Open Session 2004*

   The Secretary informed the Group of the offer of Greece to host the 2004 Session, after the option of holding the Session in Canada was no longer possible. The Session would be a very important forum to discuss the progress and results of the work programme agreed in this Session. As 2004 is an important year for the Commission, he considered that it would be appropriate to extend invitations to the Session to a wide geographical region concerned with FMD. The importance of the closed meeting to discuss the work programme was raised, and it was agreed that at least one day at the start of the Session, and a half day at the end, were necessary to achieve the required level of discussion and agreement. It was also agreed that parallel meetings should be avoided, but that pre or post-session workshops could be an effective use of the gathered expertise and opportunity. It was also recommended that the EUFMD
should take steps to ensure that member states should send more than one technical officer and that relevant scientific officers of FMD infected countries should be preferentially encouraged to attend.

4. **Media Officer and information exchange**

The Group discussed the state of information exchange between members and concluded that it would be of very considerable assistance if members were informed of press releases, opinions of members that are reported in the media, publications or reports that would be relevant to the developing policy on FMD diagnosis and control, and events of relevance including mission reports, and meetings planned. It was agreed that in the short term, Dr Griot could act as the focal point to receive and distribute such information, but that a longer term solution through the EUFMD Commission or another institution should be sought. The EUFMD Commission was urged to disseminate information on programme activities to the Research Group wherever possible. The Group was informed that the EUFMD website had been extensively improved in the last year and that it would be very important that some rules were necessary on the circulation of documents of a sensitive nature. For the longer term, it was agreed that research group position papers and answers to frequently asked questions could be a valuable addition to the website.

5. **Location of future meetings**

The gracious offer of Dr Haas to host the closed meeting in 2005 in Germany, following the move of the FMD research to the new location and laboratory to Insel Riems, was gratefully accepted by the Session.

Dr Hagai Yadin offered to host the 2006 open session in Israel. The Group acknowledged the kind offer, and discussed the level of work required to host such a meeting. The success of the previous open Session held in Israel was recalled and Dr Yadin was thanked for his enthusiasm to take on this important role once again.

**Item 12 – Open papers on emerging issues / latest developments**

Dr Bachmann presented a paper on simulation of FMD in Switzerland for contingency planning (Appendix 23). She described how the InterSpread Plus model was being adapted to the Swiss situation. The issues of spring-autumn animal movements in the Alps were discussed.

**Item 13 – Presentation and adoption of the report**

The draft report was discussed and changes adopted after discussion. It was agreed the Secretariat would propose introductory texts for several items and distribute for approval.

The meeting closed at 21.46 pm Friday.