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

Island of Moen, Denmark
12-15 September 2001

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2001
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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 on the Island of Moen, Denmark, from 12 to 15 September 2001.

Dr Per Have, Head of the Diagnostics Department of the Danish Veterinary Institute for Virus Research of Lindholm welcomed the Research Group members to the meeting. He informed them that this year is a special year in that the Institute is celebrating its 75th anniversary. It is therefore the perfect opportunity to host this important session of the Research Group taking into consideration the present FMD situation in Europe. This will no doubt put into focus this gathering. He wished all a very interesting few days in discussing different aspects of FMD. He regretted the fact that representatives from UBI were unable to attend due to the tragic situation in USA and that Nick Knowles from the Pirbright laboratory is also unable to attend.

The floor was then given to Dr Knud Borge Pedersen, Director of the Danish Veterinary Institute for Virus Research of Lindholm and the Danish Veterinary Laboratory who proceeded to welcome the Session to Denmark. He stressed how the outbreak in February in UK has reminded us that FMD is a very serious disease which can occur whenever and wherever, and new knowledge is still required, therefore this meeting is of particular importance.

He briefly described the founding of the Institute. The last outbreak of FMD in Denmark occurred in 1982/83 in 23 herds. At the beginning of the last century the disease was sporadic but in the 1920s a very large epidemic occurred. As a consequence, the Danish Government, under pressure of the Danish farm industry, decided to establish a research institute for FMD. It was intended to start by carrying out experiments and this led to the establishment of the Danish Veterinary Institute for Virus Research on the Island of Lindholm. This locality was selected in order to avoid the spread of infection. The island was subsequently purchased by the Danish State and thus the Institute became operational. The original intentions of the Institute were to establish only short-term experiments, but in 1933 there was an outbreak of Swine fever and this led to a broadening of the Institute and included other diseases. In the late 1930s the Institute also became involved in the development of vaccines.

He then briefly described the organization of the Danish Veterinary system which is part of the Ministry of Food, Agriculture and Fisheries in Copenhagen. There are two veterinary laboratories, the Danish Veterinary Laboratory and the Danish Veterinary Institute for Virus Research based in Aarhus and Lindholm. The intention is to merge these two Institutes into one unit thus becoming the Danish Veterinary Institute.

He wished the Session a useful and fruitful meeting and stressed the importance of the Research Group’s contribution to this meeting taking into consideration the present situation in Europe. He took this opportunity to welcome everybody to visit the Institute during the duration of the meeting.

Dr Yves Leforban, Secretary of the European Commission for the Control of Foot-and-Mouth disease then took the floor. He welcomed all members of the Research Group on behalf of FAO, in particular the new members. The present Group was elected by the 34th Session of the Commission held in Rome in March 2001.
He thanked the Government of Denmark, in particular, Dr Pedersen, Director of the Institute and Dr Per Have for hosting this meeting near the institute of Lindholm. The venue is particularly appropriate for such an important meeting taking into consideration the new introduction of FMD in Europe which has had serious consequences.

He explained that at the same time, there is an important turnover in the staff of the EUFMD Secretariat. Ms. Joan Raftery retired immediately after the 34th Session after 24 years with the Commission. A replacement Administrative Assistant is being appointed by FAO. For the time being, Ms Egiziana Fragiotta is covering this interim period. He thanked John Ryan, who will be leaving in early October, for his valuable contribution to the activities of the Commission during the 3-year period of his assignment. He also thanked the Government of Ireland for funding his appointment.

He then proceeded to inform the meeting of his intentions to leave the post of Secretary during the year 2002. He emphasized that he will be leaving purely for personal reasons. He considered it important to inform the Group members at an early stage in order for them to have time to think about candidates for the position. The new post description has been submitted to the Executive Committee members and the vacancy will be issued within the next few months.

He took this opportunity to thank everybody for their cooperation and help which he highly appreciated. He enjoyed working with the Research Group members and always enjoyed the organization of the Research Group meetings. He stressed the fact that he will continue to carry out his duties as Secretary until his departure and will do his best to ensure a harmonious transition with the new Secretary. He wished the members a fruitful meeting and an enjoyable stay on the Island of Moen.

It was suggested that the election of the Chairman of the Research Group be done at this stage. It was unanimously agreed that Dr Kris De Clercq be re-elected to cover the next 2-year period.

Dr De Clercq accepted the task once again. However, he wished to point out that in future the procedure for election should be changed. He suggested that anybody wishing to present their own, or to propose another candidature, should contact the Secretariat before the meeting so that the nominations can be looked at in advance. He informed the meeting that he would do his best to carry out his duties as Chairman. He thanked the Danish Government, Dr Pedersen and Dr Have for hosting and arranging this meeting and congratulated them for the 75th year of the Institute. He also thanked the Secretariat of the Commission for the work carried out in preparation for the meeting. He thanked John Ryan for his contribution to the Commission and wished him success for his future. Particular gratitude was given to Dr Leforban as his contribution to putting the Commission back on the rails has been of utmost importance. He has done an excellent job and the new Secretary will have the important task of continuing the work that Dr Leforban has carried out so efficiently. He invited the members to either put forward their own candidature for the position or to suggest suitable candidates.

He then spent a few words on the Administrative Assistant of the Commission, Ms Joan Raftery. He informed the meeting that she has probably been with the Commission for longer than most of the members of the Research Group. She was very dedicated to the Commission and carried out her duties diligently and efficiently. He spoke to her a few days before the meeting and she requested that he convey her message to the Research Group on her behalf. She regrets not being present in Bulgaria and at this present meeting. This would have been her last meeting and would have been the perfect opportunity to say good-bye to all personally. She fell ill immediately after
the last meeting in Rome. She underwent major surgery and is presently under intensive chemotherapy. She was not expecting this to occur, but is nonetheless quite serene and is doing her best to recover. Her message ended by conveying her warmest wishes to all.

Dr De Clercq took the opportunity to welcome both old and new members to the meeting of which approximately half are new members.

Prof. Reinhard Ahl of the Scientific Committee on Animal Health and Welfare of the European Commission in Brussels, conveyed the Committee’s regards to the organizers and members of the Research Group. He reminded the group of the meeting in 1990 at this location which was chaired by the late Morton Eskildsen. He recalled the last meeting in Borovets, Bulgaria when at the time, FMD had been cleared from Albania, Bulgaria, Macedonia and the risks seemed to be well assessed and under control. However, this situation had changed dramatically this year and the ongoing threat of FMD in Europe had not yet been resolved. This meeting could be considered as a special one as the contributions would provide scientific advice and knowledge to the present disease situation.

The meeting was chaired by Dr Kris De Clercq (Belgium). Members of the Group present were: Drs. Aldo Dekker (the Netherlands); Franco De Simone (Italy); Chris Griot (Switzerland); Bernd Haas (Germany); Per Have (Denmark); François Moutou (France); Vilmos Pálfi (Hungary); Sánchez-Vizcaíno (Spain); Ms Nilay Ünal (Turkey); Hagai Yadin (Israel); and, Soren Alexandersen who replaced Alex Donaldson as representative from the World Reference Laboratory. Observers attended from Turkey, WRL and the EC.

**Adoption of the Agenda**

The following Agenda was proposed for adoption.

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The Agenda was adopted as proposed.
Item 1 - General information on FMD situation in the World

John Ryan presented the FMD situation in Europe and over the world in 2001 (Appendix 1). He noted that the FMD situation world-wide has deteriorated significantly over the period 2000-2001 with different types of virus spreading beyond their traditional endemic areas.

Countries that had been free of the disease for long periods of time have had to cope with introductions of virus and the subsequent difficulties of disease eradication. Other countries that were considered to have improving situations with regard to FMD have experienced the reintroduction of the disease which abolished the advances made in recent years. The restrictions associated with the measures taken to control the disease have had severe societal and economic impacts.

Within serotype O, the PanAsian O strain has been particularly successful in spreading over long distances and affecting countries with a long history of freedom from FMD, such as Japan, Republic of Korea, Mongolia, South Africa, UK, Ireland, France and the Netherlands. This pandemic type O invaded the UK where the first cases were detected in February this year. From there the disease spread to France, the Netherlands and Ireland. Currently, all European countries with the exception of the UK have managed to eradicate the disease, either with (the Netherlands) or without (Ireland, France) emergency vaccination.

With regard to type A, massive outbreaks of disease caused by an A strain in South America have necessitated the return to mass prophylactic vaccination in Argentina and Uruguay. The complex situation with 3 distinct A strains circulating in the Middle East and Turkey continues to make control efforts difficult in this region.

Type Asia 1 continues to be endemic in South and South-East Asia, it is persisting in Turkey and Iran after its recent invasion and it continues to spread into the Caucasian countries.

Type SAT 2 has spread from its traditional endemic zone in Africa and has caused outbreaks in Saudi Arabia and Kuwait for the first time.

Nilay Ünal presented the situation of FMD in Turkey (Appendix 2). FMD continues to be endemic, with 3 serotypes (O, A, Asia 1) causing disease in Anatolia. The most prevalent serotype is type O, Asia 1 also continues to cause outbreaks, but type A was not as important as in other years, only two outbreaks were recorded and these belong to only one strain, A/Iran/96. Turkish Thrace experienced its first outbreak since 1996, with type O affecting a goat farm in Tekirdag province. The disease has been rapidly controlled by ring vaccination.

Soren Alexandersen presented a paper by Nick Knowles on the molecular epidemiology of recent type O isolates collected by the WRL from all over the world (Appendix 3). He highlighted that the interpretation of nucleotide sequence data and dendrograms should be undertaken with care due to differences in techniques and programmes employed and differences in the sections of the genome sequenced. In addition to the PanAsian type O virus, he reported that there are other genetically distinct O strains continuing to cause disease problems in South-East and East Asia, the Middle East, South America and Africa.

Aldo Dekker presented sequencing data on isolates from 24 of 26 FMD outbreaks in the Netherlands (Appendix 4). Only minor mutations were found. He concluded that the use of nucleotide sequencing remains limited in small outbreaks.
Conclusions

- Sequencing of the virus isolated in United Kingdom, France and the Netherlands indicated that the outbreaks were due to the same strain of virus.

- The FMD situation world-wide has deteriorated significantly over the period 2000-2001 with different serotypes and strains of virus spreading beyond their traditional endemic areas.

- No country is safe from the introduction or re-introduction of the disease and the risk of introduction to European countries remains significant.

- International trade in live animals (livestock, exotic pets, game species, zoo animals) and of animal products in most regions of the world is increasing. This remains the primary risk for the spread of FMD particularly because there is a general neglect of biosecurity issues and their hidden costs when driving trade liberalization measures forward.

- Improvements in roads, and in air and sea transportation increases the risk of disease spread.

- The deterioration of national veterinary services in many countries due to under-staffing, poor salaries and cut-backs in resources seriously undermines their ability to quickly uncover an exotic disease problem and respond appropriately.

- Despite the improved efforts of the Turkish authorities, the FMD situation in Turkey remains a threat to Europe.

Recommendations

- With regard to FMD, specific attention must be given to the biosecurity dangers of animal and animal product movements inherent in the structure of the animal production industry in Europe and world-wide.

- This requires that National Veterinary Services are funded and staffed to a level commensurate with their workload and responsibilities.

- All European countries should recognize the increased risk of FMD and take advantage of the lessons learned by the affected member countries to improve their contingency planning and prevention measures for FMD.

- The Commission should continue to support Turkey and the SAP Institute in their efforts to control FMD.

Item 2 - Reports on the outbreaks in Europe

François Moutou and Aldo Dekker reported on the outbreaks in France and in the Netherlands respectively, (Appendix 5 and Appendix 6) their countries and the representative from the World Reference Laboratory presented the work carried out by the Institute of Animal Health since the occurrence of the outbreak in the UK (Appendix 7). The representative from the EC also reported on the conclusions of the last meeting of the SVC on the UK situation held in Brussels on 10 September 2001 and circulated the report presented by UK. François Moutou provided additional
information on the findings of the FVO mission in which he participated as a national expert at the end of August.

Paul Barnett reported on the production of 500,000 bovine doses emergency aqueous aluminium hydroxide/saponine O1 Manisa FMD vaccine on the request of DEFRA. He described in detail the manufacture of the vaccine and the implications on the staff (Appendix 8).

Conclusions

- The FMD outbreak in UK demonstrated the important role of sheep.
- The recent epidemic had major economical consequences due to the ban of trade in all countries in Europe including free countries distant from the outbreaks.

Recommendations

- Based on the experience in the UK further research on FMD in sheep is encouraged.
- The possibility of zonification should be considered and proposals should be forwarded to OIE.
- Contingency plans should be prepared for at-risk situations or zones. EC legislation should include measures for pre-emptive culling.
- Methods for culling and disposal of carcasses should take into account the status of animals destroyed i.e infected farms, contact, vaccinated etc.
- Exchange of epidemiological and laboratory information between European countries and with international organizations including EUFMD should be encouraged. This can help laboratories in choosing the most appropriate methods for control and laboratory diagnosis (most appropriate cell and reagents for detecting a particular virus). The EUFMD should play a key role in this.
- The designation of a Community Reference Laboratory has been missing during the last outbreak in Europe. A Community FMD Reference Laboratory should be rapidly designated.
- A procedure to ensure availability of a large quantity of reagents in case of major outbreaks in Europe should be developed, possibly in cooperation with private companies. The creation of a reagent bank is a possibility.
- The situation where a major FMD outbreak occurs in the country of the laboratory designated as the Community Reference Laboratory should be foreseen.
- Methods and criteria for surveillance to regain FMD free status should be better specified in order to be included in the OIE International Animal Health Code.
- Implementation of the existing European legislations on identification of animals should be reinforced.
- There is a need for collaborative activities to be organized for virus antigen detection between FMD Reference Laboratories in Europe.
Item 3 – Reports on field and laboratory experiences during the crisis in Europe

José Sanchez-Vizacaino, Franco de Simone, Per Have, Bernd Haas and Kris De Clercq reported on the experience of their countries during the recent FMD crisis in Europe. (Respectively Appendices 9, 10, 11, 12 and 13).

The reports from all countries showed that the responses to the outbreaks in the different countries were very similar:
- Clinical inspection on farms where FMD susceptible animals were imported from countries with an outbreak;
- Serological sampling of sheep, goats and deer imported from countries with an outbreak;
- In most cases culling of imported sheep, goats and deer.

Chris Griot summerized the issue of contact with the media (Appendix 14), which was also recognized by most of the participants. There were some differences in giving a press release when having a suspicion of FMD; in most countries a suspicion of FMD was not reported to the press. The major questions arising during the crisis were about zoonosis and vaccination. Discussion on the issue of information showed that information should be given by real experts otherwise any person who is a professor or a doctor will be asked for their comments.

Several countries used the 3ABC ELISA for screening of the samples collected from imported animals, or this ELISA was used next to the standard test. Franco De Simone and José Sánchez-Vizcaíno showed that the specificity of the 3ABC ELISA (± 99.7) was superior to the specificity of the tests for antibodies against structural proteins such as the LPBE.

France De Simone presented clear data showing that in case of doubtful results a second sampling is important to clarify whether it is a specific or non-specific reaction. (Appendix 10).

Bernd Haas reported that in Germany for mass-serosurveillance using the LPBE a cut-off of 1:90 was used as in Pirbright (Appendix 12). He stated that otherwise all holdings would have been found to contain seropositive animals. Therefore, some laboratories have replaced the LPBE by the SPCE as recommended at the previous RG meeting.

It was clear that the FAO cut-off serum selected three years ago is causing problems in several laboratories. Aldo Dekker presented VNT results from slaughterhouse sera, sera from recent outbreaks and sera from four outbreaks without clinical signs. Based on these data he concluded that the cut-off related to the FAO reference cut-off serum is too low (Appendix 15). He proposed that results of serum testing should be related to the different reference sera.

The participants reported on the FMD suspicions declared in their countries. Virological examination in all countries, with the exception of the UK, the Netherlands, Ireland and France did not reveal FMD infection. Most countries used RT-PCR next to standard virus isolation. Every laboratory using RT-PCR was confident that the use of RT-PCR could shorten the time needed for diagnosis. In laboratories handling strong positive samples cross-contamination was recognized as a problem which has to be taken care of.

Reinhard Ahl mentioned that in the experience of the FMD Diagnosis Laboratory in Tubingen, BHK 21 CT cell line is particularly sensitive to the isolation of all FMD virus strains. This cell line is available from this laboratory.
In several countries parapox viruses were identified by electron microscopy in samples submitted from sheep and cattle. In a few cases BVD and MCF seemed to have caused the clinical signs. But in most negative cases, no virological cause of the signs was identified.

Kris De Clercq showed the measures applied in Belgium which were very stringent (Appendix 13). He clearly showed that before a sampling scheme is implemented one should identify the purpose of the test. A distinction has to be made between surveys looking for the presence of virus at a certain prevalence or surveys for declaring freedom of infection.

The group discussed the issue of prohibition of movement of horses during an FMD outbreak and Alf Füssel explained that within EU different systems of certification are applied between countries.

Conclusions

• Media have had a major role in the recent epidemic and a better harmonization of the messages to be addressed to the public opinion at the European level should be encouraged. EUFMD should play a coordinating role in this respect.

• Responses to the recent epidemic among different countries was similar.

• Information to the public/farmers is very essential.

• Specificity of 3ABC ELISA is superior to the specificity of tests for antibodies against structural proteins.

• The FAO cut-off serum selected three years ago is causing problems in several laboratories.

• RT-PCR is a good addition to the standard virological techniques.

• Parapox, BVD and MCF were the only virological agents identified as differential diagnosis.

• The sampling scheme implemented should be based on the purpose of sampling.

Recommendations

• Coordination of responses at an international level is needed as exchange of information on frequently asked questions.

• FMD laboratories in Europe should ensure that they use the most sensitive cells for virus isolation of all FMD strains.

• Validated tests for the detection of non structural protein antibodies and especially the 3ABC ELISA has reached a high level of performance. There is a need for reference sera for these tests.

• The LPBE should be replaced by the SPCE for detecting antibodies against structural proteins.

• Standard references for RT-PCR are necessary.

• Results of the testing serum should be related to different reference sera.
• In case of doubtful serological results, second sampling of the same animal and other nearby animals is recommended.

• FMD experts, together with epidemiologists should prepare sampling schemes for different situations in cooperation with OIE.

• There is a need for harmonization of the rules of movement of horses and of certification in Europe in the case of an FMD outbreak.

**Item 4 - Special session on new kits by private companies and IAEA**

Christian Schelp, representing Dr Bommeli AG, Intervet Company, presented data on a 3ABC test kit developed on the basis of the Brescia/WRL tests (Appendix 16). In contrast to these tests, the Intervet test does not trap the 3ABC protein by monoclonal antibodies, but purified antigen is bound directly to the plate. The indirect test employs one conjugate for ruminants and another one for pigs. The data presented indicate a specificity of more than 99% for cattle, sheep and pigs. Seroconversion in infected animals was detected between 11 days p.i. in some animals and three weeks in others. Validation data were presented for cattle and sheep indicating a sensitivity similar to that of the original Brescia/WRL test and Intervet considers the tests to be ready for marketing. However, more sera from infected pigs will be needed to complete the validation for this species.

Promising first results obtained with a new ELISA for the differentiation of vaccinated and infected cattle based on synthetic peptides were presented by Joachim Grunmach, Bayer AG. The test is based on research conducted at the Federal Research Centre for Virus Diseases of Animals, Germany (Appendix 17).

Tim Doel, Merial presented a study on the induction of antibodies to NSPs after repeated application of 10 and 20 fold overdoses of Merial vaccines. The antibody response was tested in Ingrid Bergmann laboratory by EITB. Only antibodies to the 3D NSP could be detected in significant quantity. So he concluded that no problems in respect to NSP serology would have to be feared with Merial vaccines applied according to normal vaccination procedures (Appendix 18).

As the UBI Company representative could not attend due to the tragedy in the US on 11 September, Kris De Clercq presented the data on two peptide based ELISAs he had received from UBI. One test detects antibody to structural protein VP1 (type O) and the second detects antibody to the 3B NSP of all serotypes. Data based on experiments preformed in several countries indicate that these tests are highly specific and have the potential to differentiate between FMD infection and vaccination in cattle, sheep and pigs. The test is already on the market, can be produced on a large scale and is very easy and convenient to use. Whether this test has the same sensitivity in cattle and sheep as the 3ABC ELISA needs to be further investigated (Appendix 19).

John Crowther reported on the experience of IAEA with NSP tests of various designs in many countries of the world (Appendix 20). He pointed out that available tests need more validation and better internal quality control and that also an external quality control system has to be introduced. Differences in the relative analytical sensitivity and diagnostic sensitivity and specificity of the available assays have been observed and must be taken into account when using the tests. Some indirect tests suffer from problems with certain species or individual sera giving background signals. A huge amount of data has been generated in different epidemiological situations and should be made freely available.
During the discussion Bernd Haas, Franco De Simone and John Crowther concluded that based on a comparison between the Brescia 3ABC ELISA and the UBI FMDV NS EIA, the sensitivity of the latter should be increased.

**Conclusions**

- Commercially produced complete test kits are now available. These tests have been validated extensively for cattle and also for sheep; less validation data have been generated for pigs. These tests will facilitate making use of the high throughput of regional and other laboratories normally not working with FMD. This allows to increase the testing capacity to the level required for whole herd testing of vaccinated populations. The tests are suitable for differentiation between vaccinated and infected animals on a herd basis, but will not reliably identify individual carrier animals in a vaccinated population.

- Modern purified vaccines normally will not induce antibodies to NSPs.

- Preliminary data indicate that most current tests may not be suitable for sera of wildlife species, new domestic species such as llamas, and certain breeds of buffaloes. Competition/inhibition assays may overcome this problem.

- There are differences in the relative analytical sensitivity and diagnostic sensitivity and specificity of the available assays. Further results on the performance of the existing NSP tests should be reported in the future.

- The experience of the IAEA revealed the importance of training and quality control for the application of NSP serology.

- The Brescia 3ABC ELISA has a higher sensitivity than the UBI FMDV NS EIA.

**Recommendations**

- Utilization of the existing tests for detection of NSP antibodies is encouraged for serosurveillance in Europe.

- The use of these tests in vaccinated populations should be encouraged in order to reveal cases of FMD that had not been detected by clinical inspection, increase confidence in the effectiveness of eradication measures and gather experience with the tests in various epidemiological situations.

- A reference serum bank characterizing different epidemiological situation should be established, which contains sera of relevant species in sufficient quantities for reference and developmental purposes. Further validation studies of NSP tests should be performed for pigs.

- Competition/inhibition assays suitable for sera of all species should be developed to the stage of commercially produced complete kits.

- Validation data for NSP tests, especially from South America should be made freely available.

- Further studies correlating the antibody response to structural and non-structural proteins in sera and other types of samples with virus isolation and PCR data in carriers should be performed; these parameters should also be examined in pigs.
**Item 5 - Serosurveillance**

Nilay Ünal presented a paper on the results of the serological surveillance following the vaccination in Thrace region in 2000 (Appendix 21). Sera obtained from three different sets of animals were tested by a liquid phase blocking ELISA. Although a high level of immunity was observed at 28 days post vaccination, a rapid decrease was observed in the immunity levels after 60 days onwards especially against types O and Asia 1.

Nilay Ünal presented a second paper on the results of the 3ABC ELISA serosurvey conducted with the sera obtained from Thrace (Appendix 22). In this study a total of 2,639 sera were tested. The results showed that 1% of the sera were positive. She concluded that these positive sera might be as a result of false positives or an indication of a previous infection. There are indications that some of these animals might have been introduced into Thrace from Anatolia.

Yves Leforban informed the Group that OIE guide for surveillance of FMD is being established and he circulated the draft prepared by Dr Kitching for information and comments.

**Conclusions**

- Serological results should be interpreted in conjunction with virological and other laboratory findings and with epidemiological observations.

- The serosurveillance carried out in Thrace after the 2000 Autumn vaccination campaign has been very useful. The reason why the level of immunity after 60 days and onwards was decreased rapidly should be investigated further by Turkish authorities with the support of EUFMD and EC.

- The results of the 3ABC ELISA in Thrace are favourable. They demonstrated a 1% seroprevalence and based on this result there is a low probability of circulation of the virus in the region.

- The group supported the comments made by the Secretary to draft an OIE guide on surveillance. In particular because different sampling schemes are needed depending on the purpose of the screening and this must be stressed.

**Recommendations**

- Clear guides for FMD surveillance in Europe in different circumstances combining clinical and serological surveillance should be established in coordination with OIE.

- The guides should cover different epidemiological situations vis-a-vis FMD i.e. countries or zones which are currently FMD free and wish to be recognized officially free of FMDV infection by OIE or countries or zones which wish to regain a free status after having been infected.

- The use of NSP ELISA and particularly 3ABC should be encouraged. The tests based on 3 D may also be useful in certain circumstances.

- The continuation of the Serosurveillance in Turkish Thrace using 3ABC ELISA should be encouraged and supported by EUFMD or EC.
• The comments on the OIE guide on surveillance must be forwarded to OIE.

**Item 6 – Subclinical infection and carrier stages**

Kris De Clercq reported on the carrier workshop held in the Netherlands in June. The conclusions of the workshop were summarized (Appendix 23).

Aldo Dekker reported that FMD infection in sheep can easily be overlooked and therefore additional control measures in sheep, like serological screening during quarantine, seem necessary in areas where an FMD outbreak has occurred (Appendix 24).

**Conclusions**

• Sheep are frequently subclinically infected and FMD virus can persist in this species.

**Recommendations**

• If sheep are involved in an FMD outbreak, adequate serological screening must be performed.

**Item 7 – FMD diagnostics**

Four papers were presented on FMD diagnostic methods. The first, presented by Scott Reid, was an evaluation of automated RT-PCR systems to speed up FMD diagnosis (Appendix 25). Automation was carried out in a MagNA LC (Roche), followed by PCR amplification in a TaqMan 5700 thermal cycler. With the methods described, 64 samples from the UK 2001 outbreak could be tested in a normal working day with a higher sensitivity than ELISA. The results showed that a second passage in primary calf thyroid cell culture can be avoided and therefore much diagnostic time saved.

The second paper presented by José Sanchez-Vizcaino on a new RT-PCR was based on detection of the 3D gene including a conserved Ahd 1 restriction site (Appendix 26). This allowed detection of all seven FMD virus serotypes as well as making a rapid digestion reaction following amplification. No cross reactions were detected with other Picornaviruses. The test described was evaluated on 48 different FMD virus isolates but included very few SAT serotypes.

Aldo Dekker presented a paper on the evaluation of a LightCycler based RT-PCR for the detection of FMD virus (Appendix 27). Twenty-six isolates from seven serotypes were tested. The evaluation showed that the RT-PCR is a sensitive technique. False positive reactions are mainly caused by cross-contamination with highly positive samples.

The fourth paper, presented also by Aldo Dekker, was a validation of a quick and simple monoclonal antibody-based ELISA for multi-species detection of antibodies in serum directed against type O FMD (Appendix 28). The ELISA had a specificity of 96%. The sensitivity was 98% relative to the virus neutralization test when testing cattle, pig and sheep sera collected from FMD-infected Dutch farms and scored 459 out of 484 virus neutralization test-positive experimentally derived sera correctly (95%).

**Conclusions**

• Automated RT-PCR systems with TaqMan amplification with no contamination has been evaluated. It allows 64 samples to be tested per working day. A second passage in cell culture can be avoided if RT-PCR were positive in the first passage.
• A LightCycler RT-PCR suitable for FMD diagnosis has been evaluated. It was up to 10 times more sensitive than virus isolation but contamination can still be a problem.

• The identification of the 3D gene by RT-PCR looks like a good diagnostic test for primary infections with any FMD virus serotype and the rapid digestion reaction can be used as an additional confirmation step.

• A monoclonal antibody based ELISA as a screening test for the multi-species detection of antibody to FMD virus serotype O was validated against field sera, slaughterhouse sera and sera from animal experiments in the Netherlands. The test had a lower sensitivity than the virus neutralization test.

Recommendations

• The automated RT-PCR systems need to be validated further on other serotypes and on probang samples.

• There is a need for more samples to be tested with a LightCycler RT-PCR and measures need to be taken to reduce contamination.

• The 3D gene RT-PCR must be evaluated with more FMD SAT serotypes.

Item 8 – Pathogenicity

Two papers were presented by Soren Alexandersen. The objective of the first study was to obtain data for FMD O viruses and a single type C isolate to enhance the capability of airborne virus simulation models (Appendix 29).

The collection of air samples near pigs infected with these strains has shown that the amount of virus (in TCID₅₀) emitted per pig per 24 hours was 10⁵.₈ – 10⁷.₆ for different FMD viruses (O1 and C Noville). Additionally, the results confirm that pigs compared to cattle and sheep are relatively resistant to infection by airborne FMDV.

The second paper dealt with aerosol excretion for the O UK 2001 in sheep and cattle (Appendix 30). An additional objective was to study the time course of virus load (infectivity and viral RNA) in nasal swabs, rectal swabs and in serum in order to assess transmission risks. Potential carrier status of sheep were examined at 28 days p.i. Infected sheep excreted around 10⁴.₃ TCID₅₀ / 24 hours and airborne excretion picked on a single day vary easily after infection. Virus as well as viral RNA were detected in probang samples collected at 4 weeks after exposure.

Conclusions

• The findings indicate that the risk of airborne transmission from pigs will vary depending on the specific virus isolate and the species of the recipient animal.

• According to the presented data it is confirmed that the FMD infected pigs function as amplifier of the virus. However, risk of airborne transmission to pigs appears to be low.

• The second paper provides a basis for developing a more comprehensive picture of the various transmission risks from livestock especially sheep.
Recommendations

- The risk of aerosol transmission from pigs is variable but significantly high therefore infected pigs should be eliminated as soon as possible.
- The study of airborne transmission risk should be stimulated.
- More quantitative data are needed to allow detailed assessment of transmission risk under various conditions.

Item 9 - Risk analysis and expert elicitation

Chris Griot presented a paper (Appendix 31) on the risk of importing exotic animals into Switzerland, holding them in a USDA, APHIS approved transit quarantine for 30 days before continuing their transportation into the USA. A formal risk analysis defined as a process consisting of risk assessment, risk management and risk communication was implemented at the Swiss Federal Veterinary Office. The calculated risk of introducing a false negative animal (e.g. FMDV infected animal) was estimated to be $5 \times 10^{-6}$ which is higher than the accepted probability of $10^{-6}$.

John Ryan presented a follow-up report of the expert elicitation session on the risk of introduction of FMD into Europe which was held in Borovets, Bulgaria in 2000.

Conclusions

- It was concluded that exotic animals which are foreseen for transit quarantine should be handled the same way as for definitive import. International standards of laboratory testing should be considered when interpreting test results from the country of origin.
- Expert elicitation is a good tool to evaluate risks of introducing FMD into a country and should be expanded in the future.

Recommendations

- A formal risk assessment process should be considered when importing exotic animals.
- EUFMD should continue to pursue a risk assessment by performing a detailed study of trade flows in animals and animal products and movements of people and other goods and conduct an expert elicitation.

Item 10 - Vaccines and antigen banks

The representatives from Turkey presented two papers on the FMD viruses circulating in Turkey (Appendices 32a and 32b). Information on the genetic and antigenic characteristics of these viruses was provided. Antigenic characterization of recent type O viruses and showed that although some viruses gave low r values there is field evidence that these viruses can be covered by O Manisa vaccine. Sequencing of one type O isolate from 2000 showed that this virus was in the group of PanAsia. Analysis of type A viruses isolated in 2001 revealed that these viruses were related A/Aydin/98 (homologous to A Iran 96). No virus similar to A Iran 99 have been isolated recently in Turkey.
Nilay Ünal presented a talk on the activities regarding the improvement of the FMD vaccine’s quality (Appendix 33). She gave brief information on the changes made at the Sap Institute and improvements achieved in the previous year.

A paper by Paul Barnett describing a novel formulation procedure able to extend the shelf-life of FMDV emergency vaccines was given (Appendix 34). The method involved preparing an oil vaccine with all ingredients into vials and storage of this formulation at ultra-low temperature until use. Experiments in guinea pigs indicated good long-term stability characteristics.

The Chairman asked the group to review the provisional recommendations from the World Reference Laboratory on FMD virus strains to be included in FMDV antigen banks in Europe (Appendix 35).

The information provided by Turkey on the adequate covering of O1 Manisa vaccine against the strains currently circulating in Turkey makes the need for inclusion of new type O in the bank less necessary. The group agreed that high potency vaccines against type O1 Manisa could be used. However, some members of the group were in favour of confirming this result through a challenge test.

Conclusions

- Improvements achieved at the Sap Institute were appreciated.
- The novel formulation procedure able to extend the shelf-life of FMDV emergency vaccines is a potentially useful method for storage of emergency vaccine for immediate use.

Recommendations

- Turkey should be encouraged to continue to characterize its strains and to send FMD samples to WRL to monitor the situation in the field.
- O1 Manisa and A/Aydin/98 seem to be suitable vaccine strains to be used in Turkey.
- Challenge test should be organized to assess the protection of O1 Manisa vaccine against recent isolates from Turkey.
- Utilization of vaccine with high payload antigen content is encouraged to give an adequate protection against new variants which may appear.
- The list of viral strains to be included in the banks as proposed by the World Reference Laboratory is endorsed by the group.

Item 11 - European Pharmacopoeia

Kris De Clercq reported on the meeting he attended with Group 15 V of the Eur.Phar. (Appendix 36). The proposed revised FMD monograph was discussed on 6 June. Several proposals made by EUFMD were taken into account. A new revision will be prepared by the Eur.Phar.

He also reported on a meeting with the CVMP/Immunologicals Working Party of EMEA. EMEA will organize a meeting on 19 September bringing together EMEA, EUFMD, OIE and EC.
The purpose will be to draft guidelines on safety, quality and efficacy of FMD vaccine production and on the introduction of new FMD strains.

Kris De Clercq explained that monographs cover vaccines for one species and there is no current monograph for pig breeding. Therefore, the question of vaccines to be used in pigs was briefly discussed by the group. Some members of the group were of the opinion that vaccines which passed the test in cattle can also be valid for pigs.

**Recommendations**

- The group recommended to continue the efforts in this field and to call on the EUFMD working group whenever necessary.

- To request the manufacturers to provide data obtained after vaccination of pigs.

**Other items**

John Ryan reported on the workshop on FMD simulation exercise held in Brno, Czech Republic from 5 to 7 June 2001 (Appendix 37).

Franco De Simone reported on the new Reference Laboratory for Vesicular Diseases at the IZSLE in Brescia, Italy (Appendix 38). The Chairman stressed the need of a laboratory for vesicular diseases in Italy and expressed the full support of the Research Group.

Yves Leforban informed the Group of the new EUFMD/EC project which is being signed. The project of US$1.5 million covering a period of 4 years intends to better define the activities supported by EC under the Trust Fund 911100. This includes support to the control programme in Turkey and in other countries where the situation is at risk for Europe and normative activities such as the organization of meetings and workshops (including the Research Group Sessions).

He also informed the Group of a request received from a small camelid breeder association in UK asking for advice to vaccinate their animals in order to avoid slaughter in the case of FMD. He had replied to this request by explaining that although scientific evidence exists that camelid are not very sensitive to FMD, the disease may be observed in camelid. This occurred recently in Mongolia and if special measures are applied to camelid they should also be applied to other species considered as low sensitive.

Nilay Ünal from Turkey confirmed the intentions of Turkey to host the next meeting of the Research Group in Izmir. The provisional dates are from 18 to 20 September 2002. Chris Griot confirmed the intentions of holding the Research Group meeting in Switzerland in 2003.

**Adoption of the Report**

The draft report of the meeting was discussed by the Session and accepted with some amendments.

**Closing remarks**

The Chairman thanked the Government of Denmark on behalf of the Research Group for the kind hospitality offered. In particular, appreciation was expressed to Dr Pedersen and Dr Per Have of the Lindholm Veterinary Institute for all arrangements made for the smooth running of the meeting. Thanks were also extended to the members for their contributions.