EUFMD/RG/05

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 (EUFMD)

held at

Greifswald, Insel-Riems, Germany

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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INTRODUCTION

A closed 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 Insel-Riems, Greifswald, Germany from 20 to 23 September 2005.

Members of the group present were: Drs. Sinan Aktas (Turkey); Soren Alexandersen (Denmark); Emiliana Brocchi (Italy); Mark Bronsvoort (UK); Kris De Clercq (Belgium); Aldo Dekker (the Netherlands); Georgi Georgiev (Bulgaria); Matthias Greiner (Germany); Bernd Haas (Germany); François Moutou (France); Dónal Sammin (Ireland); Hagai Yadin (Israel) and David Paton, *ex-officio* (UK).

Observers present were Dr Alf-Eckbert Füssel, DG-SANCO, EC Brussels, Dr Carsten Pötzsch of the Friedrich Loeffler Institute; Dr Francis Geiger, Central Asia FMD Surveillance Project, Iran; Dr Manzoor Hussein, based in Pakistan, Regional Epidemiologist of the FAO project GTFS/INT/907/ITA; and Dr Erika Carlsson, FAO Tajikistan. David Paton represented the OIE, Paris.

The EUFMD Secretariat was represented by Dr Keith Sumption (Secretary), Dr Thomas Murray (Associate Professional Officer) and Ms Egiziana Fragiotta, Administrative Clerk.

The formal welcoming address from the German Authorities was given later during a visit to the Friedrich-Loeffler Institute which organised and hosted the Session. The Chief Veterinary Officer of Germany, Dr Karin Schwabenbauer, was represented by Dr O. Hauck. He conveyed the apologies and best wishes of CVO who could not be present to welcome the participants personally due to other commitments. He assured the meeting that although in recent weeks avian influenza has taken a prominent place in Germany's media reporting, the German government has not been distracted from its monitoring of FMD. The development in Siberia where FMD seems to have spread further during the past weeks is cause for some considerable concern. Germany is in a fortunate position of having been free from FMD for some 17 years. Although this is a good overall situation, there are still a number of risks which need to be monitored. For example, lack of experience in clinical diagnosis and limitation of combating disease control through simulation exercises only. This could hinder the response to a real outbreak in terms of timeliness, rigour and effectiveness. He expressed gratitude to the EUFMD Commission for taking the initiative to develop a training model to focus on these key areas. He commended the Group on the progress made in FMD diagnostics which is vital in contemporary approaches to preventing animal disease. The shift in social expectations that surround effective animal disease prevention will, in future, have to deal with questions regarding the use of pen-side tests as well as monitoring activities as a follow-up of emergency vaccination measures. In addition to this, focus will also be placed on vaccination-to-live measures.

He concluded by stating that he is confident that this session will bring us closer to finding solutions to the problems faced. He wished them all fruitful discussions and an enjoyable stay in Germany and at the Institute.

The President of the Friedrich-Loeffler Institute, Professor Thomas Mettenleiter, also welcomed the meeting to the Institute and gave a presentation on the history and future of the Institute. The research institute was established in 1910 and is considered the oldest institute in the world founded specifically for research on virus diseases. Research on a new group of subviral pathogens has become an additional focus of research at the institute. The importance of virological research on the Isle of Riems is reflected by the fact that over the next few years significant investments will be made to establish new research facilities at the site. Demolition work is already underway and construction work will start in the near future. The plan is for work to be complete by October 2010 when the Institute will celebrate its 100th anniversary.

Kris De Clercq and Keith Sumption conveyed, on behalf of all present, gratitude to Prof. Mettenleiter and his staff for the excellent organization of the meeting and the kind hospitality offered.

Item 1. Election of the Chairman

Keith Sumption explained the procedure for election of the Chairperson, and called for nominations. Aldo Dekker nominated Kris De Clercq, seconded by Dónal Sammin. No other candidates were nominated, and by unanimous show of hands Dr Clercq's election was confirmed. Kris De Clercq thanked the group for their support and indicated that this period would complete 10 years in the Chair.

The meeting was subsequently chaired by Kris De Clercq.

As newly re-elected Chairman, Kris De Clercq welcomed the Research Group members to the session, in particular, the new members of the group. He briefed them of the meeting schedule of the EUFMD Commission and the scope of the sessions of the Research Group. He stated that the information gathered from the group is an important means of looking into the different situations in the world and the expectations of the member countries from the Group were high. This session will also look into and revise the action plan made for the 2003-2005 biennium and will prepare the plans for 2005-2007.

Item 2. Agenda

The Agenda was adopted as proposed **(Appendix 1)**. David Paton suggested the subject of co-ordination between the Community Reference Laboratory (CRL) annual meetings, and the future EUFMD Research Group Sessions be discussed. Alf Füssel suggested that some activities could be taken forward under the CRL, and that detailed co-ordination may be best achieved when the annual work plan of the CRL was available for discussion. Keith Sumption suggested that the differences between CRL and EUFMD Research Group could also lie in the latter's need for technical support related to surveillance and control actions.

Item 3. Update on the EUFMD Commission

Keith Sumption gave a presentation on the activities of the Commission and the action plan for the period 2005-2008 **(Appendix 2)**.

Kris De Clercq provided a reminder of the workplan and workgroups in the 2003-5 workplan of the Research Group **(Appendix 3)**.
Keith Sumption reported that the General Session gave a very strong acclamation of the work of the group and some members were extremely impressed by the evidence of co-operative effort to resolve technical questions.
Aldo Dekker felt it was overambitious, but looking back it was clear we had managed to do a lot of good work.

Item 4. FMD risk and the review of priority antigens

FMD Risk and Priority Antigens

David Paton gave an update on FMD risk and priority antigens for European vaccine banks **(Appendix 4)**. In 2005, there were no extensions of the disease into officially FMD-free countries that do not practise vaccination. In South America, new zones that are FMD-free with vaccination have been recognised in Argentina and Columbia and Paraguay has also regained this status nationally. A recent outbreak of FMDV type C in East Africa is probably attributable to vaccine escape. There are positive signs of increased intensity of FMD control measures in India and China, which contain the largest populations of FMD susceptible animals in the world. Despite these positive developments, there has been an upsurge in cases of FMD caused by the Asia 1 serotype with the virus being recovered in 2005 from outbreaks in China, Russia and Mongolia, following recoveries in 2004 from

outbreaks in Tajikistan, Iran, Pakistan and Afghanistan. In South East Asia, the Asia 1 virus has been reported for the first time in several years, whilst type A virus has spread further east than previously. Despite this setback the newly established network of OIE/FAO FMD reference laboratories and recent exchange visits with the Chinese National FMD Reference Laboratory has led to improved cooperation in global strain typing. The FAO world reference laboratory has developed close collaborations with laboratories in West and East Africa.

Conclusions

1. There are gaps in the tracing of the spread of the Asia 1 virus and under-reporting of outbreaks may have contributed to its wide dissemination in 2005.
2. Recent efforts to improve our knowledge of the range of FMD viruses in circulation globally have been facilitated by the establishment of new international contacts and collaborations.
3. Serotype O remains the most prevalent and serotypes A and SAT 2 the most problematic for vaccine selection due to the existence of multiple antigenic variants. Latest isolates from Iran are suggesting a return of A viruses antigenically similar to A 22 Iraq.
4. Vaccine recommendations remain unchanged, and the Shamir vaccine is expected to provide coverage against the current Asia 1 outbreak strains.
5. Inadequately inactivated vaccines still contribute to the pool of circulating viruses in some parts of the world.

Recommendations

1. FAO should continue to encourage cooperation and exchanges between the WRL and collaborating laboratories in Africa and Asia.
2. The WRL should seek to obtain candidate vaccine strains and antisera in order to look for matches against viruses for which existing vaccines provide little predicted cover.
3. FAO should support studies by reference laboratories to compare their vaccine matching methods, including the exchange of vaccine viruses and vaccine antisera. This will provide more confidence in vaccine strain selection.
4. The EUFMD Research Group should prepare a paper outlining the case for ceasing vaccination against type C and recommending any accompanying safeguards that will be needed.
5. FAO and OIE should organise a meeting of the Steering Committee of the OIE/FAO network of FMD reference laboratories at which issues of support to the network, the involvement of additional laboratories, and the co-ordination of international efforts to improve sample submission be addressed.

Item 5. Regional risk situation

Risk of FMDV types Asia 1 (and others) from Central Asia to Turkey

Manzoor Hussein gave an overview on major animal disease situation in Pakistan, Afghanistan, Uzbekistan, Tajikistan and Turkmenistan **(Appendix 5).** FMD is endemic in the Central Asian region with different virus strains circulating and insufficient capacities for surveillance and diagnosis. Difficulties in samples shipment for diagnosis have been experienced. To increase the efficiency of a regional cooperation a better notification of outbreaks and exchange of data is to be encouraged. Borders are porous throughout the region making regional approaches to disease-control necessary. The Indian-Pakistan border will become even more open as relations between these two countries improve. In Pakistan no national system for disease recordings is available today. Local vaccine production is not sufficient.

Erika Carlsson added useful comments on the situation in Central Asia stressing the importance of a better global transparency.

Francis Geiger presented three papers on his project with IVO in Iran **(Appendix 6)**. FMD diagnosis data in Central Veterinary Laboratory (CVL) of Iran during the last 4 years have been presented with details about O, A and Asia1 virus strains evolution during this period. CVL (as Iranian NRL), uses as routine methods: ELISA antibody detection, cell culture and RT-PCR. FMD outbreaks which occurred during the last 5 months and related FMD diagnosis with mapping localisation have also been presented for better understanding of the current situation, particularly along the borders with Turkey, Afghanistan and Pakistan.

The EUFMD project of Central Asia FMD surveillance centre ongoing in Iran will allow a better knowledge of virus circulation in the region.

Sinan Aktas presented a paper on the surveillance conducted in Turkish Thrace **(Appendix 7)**. A total of 9728 sera collected from 152 villages were tested for antibodies against NSPs. During the follow up study 42 positive animals and an infection in a village near Istanbul dating from early 2005 was discovered. Except for this village, there was no other evidence that there has been no active virus circulation in Thrace.

A summary of the activities in Erzurum region, Turkey since 2003 was presented by Tom Murray and Sinan Aktas **(Appendix 8)**. Particular reference was given to the visit in June 2005 and the current participatory study being carried out there from early August to the end of September. Preliminary data were presented on the epidemiological investigation. Four active FMD outbreaks have been detected so far in the province.

Conclusions

1. Information exchange about FMD in central Asia including Afghanistan and Pakistan is important for EUFMD member countries.
2. Such information is still incomplete for several different reasons.
3. The design of the survey including follow up provided useful data on the situation in Thrace in 2005.
4. Erzurum study may prove participatory epidemiology can be used as a tool for identifying diseases situations in a region and can complement classical FMD investigation.

Recommendations

1. The FAO and OIE animal health projects working in this geographical region should seek to improve exchange of information including increased detailed surveillance and reporting, including identification of species, virus strains, number of animals, precise location of clinical disease and virus isolation, etc. focusing on border areas.
2. The EUFMD should seek to support FMD control and surveillance activities in countries east of Turkey.
3. FAO regional projects should facilitate the sharing of information on FMD control in high risk border areas.
4. To facilitate maximal output of ongoing project (central Asia FMD surveillance centre, GTFS/INT/907/ITA, FAO Tajikistan, EU Afghanistan, EU Pakistan) including easier access to laboratory diagnostic facilities through common workshops. EUFMD could function as an umbrella for such increased cooperation.
5. Turkey should be encouraged to continue to organise sero-surveillance in Thrace region and EUFMD should continue to support these studies.
6. Turkey should be encouraged to extend active surveillance studies in Eastern Anatolia.

Item 6. Review of support required to FMD Laboratories for quality assurance

David Paton described a pilot study **(Appendix 9)** for external quality assurance of virus isolation and RT-PCR, in which a proficiency panel of samples containing live FMDV were distributed to four European Reference Laboratories. For serological quality control, a panel of 38 bulk volumes of bovine sera has been established to evaluate the sensitivity of new NSP tests and to control for the quality of new batches of existing tests. Future priorities for serological test standardization and proficiency testing were discussed.

Georgi Georgiev presented an evaluation **(Appendix 10)** of the range of laboratory diagnostic tests currently used by Western Balkan, Former Soviet Union including Transcaucasian countries, along with their needs and desires with regard to proficiency testing. Some countries do not have sufficient access to diagnostic reagents. Many do not have systems of quality accreditation in place and have not yet participated in proficiency tests.

Conclusions

1. The pilot study revealed differences in the sensitivity of virus isolation systems, even where apparently similar cell lines were in use.
2. The proficiency panel tested in five laboratories will be useful for wider use, but the specificity of tests should also be checked as should the performance of the antigen detection ELISA.
3. Western Balkan, Former Soviet Union including Transcaucasian countries mainly rely on antigen detection ELISA, liquid phase blocking ELISA plus or minus NSP ELISA for their diagnostic needs.
4. Next year's proficiency testing will concentrate on a wider distribution of the existing live virus proficiency panel used in the pilot along with a distribution of a serology and antigen detection ELISA panel based on inactivated materials.
5. The serology proficiency panel should be applicable to both non-structural and structural protein antibody tests and the priority serotypes are O, A, Asia 1 and SAT 2.
6. The WRL NSP serum panel could be used by reference laboratories on behalf of national authorities to safeguard the sensitivity and equivalence of large batches of NSP reagents that are being established as contingency reserves.

Recommendation

1. Future proficiency testing should include all EUFMD member countries as well as neighbouring states, but first of all, the laboratories need to establish the relevant tests in use. One category of laboratory that cannot receive infectious virus should be provided with inactivated antigens and sera for evaluation of ELISA methods for virus detection and serology. Other laboratories able to receive infectious materials also require proficiency panels for evaluation of virus isolation and molecular detection methods.

Item 7. Progress on technical questions relating surveillance post-emergency vaccination
Post e-vaccination

Progress on technical questions relating to surveillance post-emergency vaccination

David Paton presented an update on OIE activities and the EU coordinated action on FMD and CSF **(Appendix 11)**. The OIE ad hoc group on antigen and vaccine banks has decided on a new general chapter on vaccine and antigen banks, as well as a revised and a new section in the existing FMD chapter regarding FMD vaccines and vaccine matching respectively. The EU coordinated action and the OIE Ad Hoc Group on FMD antigen and vaccine banks is establishing a network of FMD vaccine bank managers and one for OIE/FAO FMD reference laboratories. The OIE ad hoc group on NSP tests will meet in January 2006 where data on pigs and sheep will be considered. Suggestions made by the EUFMD RG for modification of the PVS guidelines were not adopted. The OIE position was that requests for modification must come from a member country. There is a new two-year cycle leading up to adoption of new or revised documents by the general assembly. The OIE has secured

amendments to reduce the stringency of the IATA regulations for transport of diagnostic specimens; this should facilitate submissions to OIE reference laboratories.

Conclusion

1. The mechanism for executing a change in the OIE guidelines on post-vaccination surveillance for FMD is now clear and the RG could offer guidance if changes are required.

Recommendations

1. FAO/OIE should provide funding to establish a network of regional reference laboratories.
2. The EUFMD executive committee should consider if there is any need to change the current OIE guidelines and terminology to better reflect the European approach to PVS.
3. The revised IATA regulations for transport of diagnostic specimens should be incorporated in the updated EUFMD position paper on sample transport.

Progress report on NSP test validation and gaps remaining

Emiliana Brocchi presented a finalised analysis of the data generated at a workshop held at IZS, Brescia in May 2004 on comparative evaluation of NSP antibody detection ELISAs (NSPEs) **(Appendix 12).**

Conclusions

1. There is now sufficient data from vaccinated cattle for reliable estimation of the diagnostic sensitivity and specificity of the available tests.
2. One of the NSPEs now commercially-available in Europe, the Ceditest, and an in-house test developed at IZSLER, Brescia, perform as well as the OIE reference method from Panaftosa.
3. The workshop has therefore provided data which can be used in the design and interpretation of PVS.

Recommendation

1. Studies to address knowledge gaps in validation data from sheep, goats, water buffalo and pigs should be supported, in particular data should be generated on the specificity of NSPEs in vaccinated animals of those species.

Use of NSP for detecting infection in vaccinated pigs in Hong Kong

Dónal Sammin presented data on the use of NSPEs for detecting infection in vaccinated pigs, **(Appendix 13)** based on specimens collected in the Hong Kong Special Administrative Region (HK-SAR). The presence of hoof lesions was found to correlate with NSP AB+. Pigs without hoof lesions did not develop NSP antibody responses.

Conclusions

1. The findings support that clinical disease in pigs might be required to generate a good antibody response to NSP.
2. The relative sensitivities of the UBI "testing system" and the Cedi test were equivalent and both were better than the Bommeli CHEKIT ELISA.
3. The specificity of the UBI "screening" test was lower than the other two NSPEs but was improved after application of the UBI "confirmatory" test.

Post-vaccination surveillance guidelines and their application

David Paton described some ways in which NSPEs **(Appendix 14)** could be used in combination and the consequences on diagnostic sensitivity and specificity at the herd level. He emphasized that serosurveillance can substantiate rather than demonstrate freedom from infection after an outbreak. Different combinations of tests can be used in series to improve the overall diagnostic specificity but sensitivity remains a limiting factor in achieving the required level of confidence within small herds.

Conclusions

1. Probang-sampling followed by RT-PCR is insufficiently sensitive unless three serial samples are tested.
2. Possible solutions to the "small herd problem" include: (i) not vaccinating them; (ii) vaccinating them but increasing the number of small herds "sampled" to increase the probability of detecting infection **or** (iii) applying a vaccinate-to-kill policy.

Recommendations

1. The algorithm combining initial cedi test, retest of positives by cedi and final confirmation by the svanova ELISA currently provides the best available serodiagnostic system within Europe, with respect to diagnostic specificity after vaccination. Combinations, including in-house tests proven to provide equivalent performance, may also be appropriate.
2. Competent authorities should be advised that it is not possible to reach the required level of confidence when testing small herds.
3. If vaccination is not used, the combination of the Ceditest (or other equivalent NSP test) as a screening test and SPCE as a confirmatory test can do away with the necessity to handle live virus in performing the VNT.

Designing post-vaccination surveillance

Matthias Greiner presented a new software tool **(Appendix 15)** to optimise herd sensitivity whilst maintaining minimum herd specificity by altering the numbers of samples taken per herd and the cut point at which a farm would be considered positive. He explained with regard to serial testing it is advantageous to select tests that are non-covariant with regard to specificity and covariant with regard to sensitivity. He modelled two scenarios for serial testing using herd population data from Denmark.

Conclusions

1. A combination of serial testing with a high specificity can still result in a high herd level sensitivity although the sensitivity at individual animal level is lower.
2. The current NSPEs are fit for purpose to substantiate freedom of FMD at a 5% prevalence at herd level and 2% prevalence between herds.
3. Further work is required to refine and validate the proposed tool by modelling the uncertainties of the data.

Recommendation

1. The economic advantage of this optimised sampling strategy should be compared with the more conventional approaches.

Use of likelihood ratios in analysis of NSP test results

Dónal Sammin presented the possible use of likelihood ratios **(Appendix 16)** in interpretation of diagnostic test results and provided a means of combining results obtained with two of more tests.

Likelihood ratio can be used to revise pre-test odds of infection, but this approach requires that pre-test odds can be quantified and explicitly stated.

Conclusion

1. More thought needs to be given to the manner in which likelihood ratios are used.

Recommendation

1. A combined likelihood ratio should be calculated for the number of seropositives in a herd and the magnitude of the test result; this should be calculated using field data.

Alternative view – application of NSP

Aldo Dekker determined the number of sera **(Appendix 17)** that would have to have been tested during the 2001 FMD outbreak in the Netherlands, if the surveillance provisions of the 2003 EC directive had been in force at that time and a vaccination to live policy had been enforced within 2 km of each infected premises. Assuming the absence of infection, approximately 10% of the vaccinated farms would have tested positive after two rounds of herd sampling. A decision tree was presented based on a previous EC directive 200/428/EC on control of SVD; according to this approach singleton seropositive animals would be culled and the herd would be retested.

Conclusion

1. Within seropositive herds, slaughter under controlled conditions is the preferred method of dealing with seronegative animals whilst the seropositives would be culled.

Overall Recommendations

1. The 3 inter-related issues raised in these papers should be dealt with separately, i.e. (i) demonstrating freedom from infection, (ii) defining what PVS findings would constitute a new FMD outbreak and (iii) removing animals from seropositive herds to mitigate risk.
2. The risk remaining after applying different surveillance strategies should be evaluated.

An EUFMD workshop on design and interpretation of post vaccination serosurveillance

Objectives
Given the current FMD-free status without vaccination in Europe and the possibility of a future outbreak with vaccination-to-live used as an emergency measure and being followed up by post vaccination serosurveillance to return to the favoured status of free without vaccination:

(1) design and implementation of a serosurvey: (a) to detect infected herds/flocks or (b) to prove freedom from infection
(2) how to interpret and follow-up seropositive animals and/or herds/flocks
(3) how laboratory test results can be used for rational decision-making
(4) to identify the resources required to undertake the preferred strategy

Participants

(1) Decision-makers: CVOs, heads of NDCCs and others
(2) Technical: laboratory-based experts and veterinary epidemiologists
(3) Representatives of DG-SANCO and OIE

Timing: Spring 2006
Venue: To be defined

Outputs: Report which will assist the working group to finalize guidelines for specific epidemiological situation.

Item 8. Sero-monitoring of virus circulation and FMDV vaccination in the Caucasus

Three presentations were made by Carsten Potzsch, Emiliana Brocchi and Matthias Greiner on the serosurveillance conducted in the vaccination buffer zone in the trans-Caucasus region (Armenia, Azerbaijan and Georgia). (**Appendices 18, 19 and 20).** The objectives of the serosurvey were to estimate the level and describe the geographical distribution of antibodies to structural (SP) and non-structural proteins (NSP) and to interpret the findings in relation to the effect of vaccination and evidence for circulating infection. In the buffer zone the trivalent vaccine produced by ARRIAH, Russia was used in the previous two years. In Armenia locally produced lapinised vaccine is still used in the country outside the buffer zone. In Georgia a similar vaccine was used until spring 2004 since then purified vaccine is used. In Azerbaijan only vaccine produced by ARRIAH Russia has been used in previous years. The survey was designed as a two stage (selection of villages and animals within the village) random sampling with emphasis on young animals. The study was aimed at the detection of 10% intra-herd and 10% inter-herd prevalence. Thirty villages were randomly selected in the buffer zone in each country in which 30 animals were selected. About 900 sera were collected in each country and analysed in the FAO Collaborating Centre for Vesicular diseases in Brescia, Italy, for the titration of antibodies against FMDV O Manisa, A Iran 96, Asia 1 (solid phase blocking ELISA, Brescia tests) and antibodies to NSP (3 ABC-ELISA, in-house assay).

The test results were analysed using geographical information system and descriptive statistics. For Armenia, the overall sero prevalence for O, A and Asia were 83% 92% and 93% respectively. The figures for Azerbaijan were 69%, 93% and 90% and for Georgia 47%, 42% and 34%. In Georgia the village level SP prevalences were highly variable whilst in Armenia and Azerbaijan the distribution was more homogeneous and positive sera scored high antibody titres.

The overall NSP prevalence for Armenia was 15%, for Azerbaijan 8% and for Georgia 3%. The distribution gave evidence of spatial clustering. There was no evidence for a relation between age and SP nor age and NSP sero-positivity. In some villages NSP sero-reactors including young animals, showed high titres against O or A serotypes which could be an indication of circulation of those virus types. There was no association between age and NSP seroprevalence, suggesting that repeated vaccination with unpurified vaccines cannot explain alone the finding of NSP sero-reactors.

Conclusions

1. In the animals sampled in Armenia and Azerbaijan the vaccination was generally effective in inducing high antibodies titres, irrespective of the age of the animals.
2. The results are consistent with, although do not provide proof of, current or recent virus circulation in the three countries. Assuming that the vaccination effect to elicit NSP response is identical in all study regions, the observed heterogeneity in NSP positivity rate may be interpreted as an indication of circulating infection in some counties.

Recommendations

1. Follow up investigations should be conducted in villages where positive NSP results were found with emphasis on young stock.
2. Investigations should be extended countrywide to provide baseline information. Follow up seromonitoring should include other susceptible species and should be risk based.
3. The results should be discussed with national veterinary services and regional cooperation on disease and surveillance and control should be strongly encouraged.
4. Cooperation with European institutions/laboratories on epidemiology and diagnostic support should be continued.

Item 9. Developments in FMD control decision support systems

Mark Bronsvoort presented a paper on decision support systems **(Appendix 21).** Decision making is becoming more difficult and complex with increasing availability of information and increasing demands from stakeholders and the general public to get every decision right. Decision support systems (DSS), which are usually computer based, offer the ability to manage large amounts of data and produce descriptive summary reports. Incorporation of mathematical models, GIS and economic models has greatly increased the potential to use these systems in planning for epidemics, risk mapping and resource allocation as well as exploring different strategies such as stamping out, vaccination and combinations of these.

The use of mathematical models during outbreaks to predict spread has proved more controversial. Some comparisons are given between the competing models and attention is drawn to the new model by 'Risk Solutions', which as well as being explicitly spatial also includes a novel intra-herd spread component.

	Imperial	***Edinburgh/ Cambridge***	***Interspread***	***ExoDis (Risk Solutions)***
No. parameters	***few***	***few***	***many***	***Some***
Spatially explicit		✔	✔	✔
Different species		✔	✔	✔
Airborne spread			✔	✔
Different transmission mechanisms			✔	✔
Intra-herd transmission dynamics				✔
Logistic/resources			✔	✔
Vaccination strategies		✔	✔	✔

Conclusion

1. These DSSs have a very important role in many areas of decision making including risk assessments, contingency planning, identifying high-risk farms or dealers. However, it should be emphasised that they are support systems and that mathematical models in particular should be interpreted with great care as many of the assumptions are not explicit and the output can appear very seductive without making any of the uncertainty explicit.

Recommendations

1. Efforts should be made to combine trade and animal movement data across the EU with data on the global occurrence of FMD and to incorporate this information in risk assessment systems to allow rapid and consistent decision making.
2. Effort should be made to identify gaps in knowledge where models could be usefully applied such as in the area of transmission of infection and different sizes of vaccination zone to compare likely costs and resource requirements.
3. Efforts need to be made to assess the epidemic and economic models particularly in the light of the new Risk Solution model and identify any data sets that might be appropriate to validate and understand how these models might be better used in epidemic planning and during outbreaks.

Item 10. Laboratory bio-security

Bernd Haas presented a draft **(Appendix 22)** of "Minimum standards for bio-security for laboratories situated in FMD infected countries or zones undertaking the testing of samples from holdings with clinical signs indicating the possible presence of FMD". These standards were intended for laboratories undertaking the testing of samples from holdings with suspect cases of FMD without propagating FMD-virus in cell culture or animals. This has become possible after methods (ELISA, RT-PCR) have become available which are based on techniques which do not require live FMD virus. Whereas FMD free countries and traditionally FMD free countries attempting to recover the free status after an outbreak should test samples from holdings with clinical signs indicating the possible presence of FMD only in laboratories meeting the "Security Standards for FMD laboratories" adapted by the EUFMD General Session in 1993", the special buildings and equipment required for such FMD laboratories are expensive and difficult to maintain. In FMD free countries the costs are justified because laboratories capable of employing the full range of diagnostic methods and doing research are an indispensable component of modern disease control whereas any escape of virus from such a laboratory could have catastrophic consequences. However, in endemically infected countries, the risk of virus escaping from a laboratory without such advanced bio-security features has to be balanced against the advantages of the availability of a diagnostic laboratory in relatively close proximity to the site of outbreaks. Still, the risk of virus escaping from a laboratory has to be mitigated by appropriate provisions. The draft presented is based on the "Minimum standards for bio-security for laboratories undertaking serology with blood samples from areas not considered free from foot-and-mouth disease" adopted on the EUFMD RG session in Chania in 2004. In particular, such laboratories are not required to have negative pressure and HEPA – filters.

Conclusion

1. Under special conditions it will be of advantage to allow laboratories not meeting the security standards for FMD laboratories adopted in 1993 to carry out the laboratory diagnosis of FMD with methods which do not require the propagation of virus. However, these exceptions should not compromise the efforts to exclude the escape of FMDV from laboratories in FMD – free countries.

Recommendations

1. A working group should study the differences between the FAO guidelines for FMD labs and the requirements of the OIE containment group IV and prepare a proposal for a possible revision of the OIE requirements.
2. The possibility to diagnose FMD with samples inactivated on the premise or using pen– side tests should be further investigated.

Item 11. Virus inactivation studies – progress report

Soren Alexandersen gave a short summary **(Appendix 23)** of the known data on FMDV inactivation kinetics in milk and milk products and did in particular describe the uncertainties inherent in the limited experimental data available. His presentation pointed at the uncertainties in relation to minimum doses to infect (in particular in animals with cuts or abrasions in the oral mucosa), excretion levels in individual animals in combination with in-herd and between-herd prevalence a time of reporting as well as uncertainties in virus inactivation kinetics in milk and milk products under various conditions and influenced by e.g. cellular content or lipid and protein concentration, pH etc.

Matthias Greiner presented the preliminary results of a study **(Appendix 24)** directed at describing what is known about FMDV survival in meat and meat products and the input required for such an analysis from a risk assessment point of view. The framework for a risk assessment was listed as were known knowledge on FVDV inactivation in meat and meat products. The knowledge of pH

development in pork was mentioned and it was emphasised that the data on FMDV inactivation in pork was very limited.

Aldo Dekker described the preliminary results **(Appendix 25)** of a risk assessment on the risk of FMDV in pork from vaccinated animals. The scenario tree for the analysis was demonstrated and the background and rationale for the semi-quantitative risk assessment described. The results of the assessment using various assumptions suggested that the resulting risk of FMDV in pork from vaccinated pigs was low, however, it was acknowledged that gaps exist in data available for estimating various parameters and therefore, that the uncertainties inherent in the assessment is significant.

Conclusion

1. Clearly, more studies, at both the experimental and theoretical levels, are needed in order to provide relevant estimates of the risk of spread of FMD by milk and milk products and by meat and meat products in relation to both the period before disease is reported and, in case vaccination is performed, the period from sero-surveillance has been initiated and freedom from infection has been achieved.

Recommendations

1. A working group should immediately be established and urgently draft detailed plans for further studies in collaboration with relevant partners including the industry.
2. Funding and input from industry as well as possibly FAO and EC should be sought.
3. A suggested proposal for samples that may be required from countries with endemic FMD could be forwarded to FAO for potential action and it should be considered if large parts of the studies could be performed in countries with endemic FMD.
4. The studies should be designed so they can provide D and Z-values of FMDV inactivation in products of interest and should be done under conditions making comparison to earlier studies feasible. The studies should focus on potential differences in inactivation kinetics in various fractions of the product, e.g. the cellular fraction versus the skim milk or cream fraction, and should also provide further data on the actual dose needed to establish infection by the oral or aerosol route by relevant products, in particular in animals with abrasions or cuts in the oral epithelia.
5. It is recommended that relatively large scale studies are done in order to provide statistically significant data.

Item 12. Risk assessment/management papers for review

François Moutou presented an extensive review **(Appendix 26)** of the role wildlife might have in an outbreak of FMD under European conditions. Most species of free-ranging wildlife and zoo animals, except species from the family of bovidae and suidae, are based on available evidence likely to have only a minor or negligible epidemiological role.

Soren Alexandersen presented the results **(Appendix 27)** of two FMD experimental infection studies in dromedary camels in Dubai. Only cattle passaged FMD virus type O was able to infect 1 out of 5 camels and no infection was observed in 5 contact camels and in 4 contact sheep. More work will be done with this type O virus and an other type A strain.

Hagai Yadin added some information on infection in gazelle and wild boar in Israel. Although a high proportion of gazelle have been seropositive after being involved in FMD outbreaks, the prevalence of antibodies dropped indicating that the virus did not circulate on its own within the gazelle population.

Aldo Dekker presented the results **(Appendix 28)** of a meta-analysis used to quantify the transmission rate from FMD carrier animals. The analysis showed that the risk to infect cattle is very low, but the risk of infection of other susceptible species might be 11% per month per carrier. Sensitivity analysis

showed that removing the data of positive result would not change the outcome very much. Analysis of the percentage of carrier cattle after infection showed on average 61% carriers at 28 days post-infection with a half time of about 6.3 months.

Conclusions

1. In all recent European outbreaks wildlife were not implicated in the spread of FMD and the outbreaks could be controlled without any specific action devoted to wild species, native or exotic. This extensive review of the role of wildlife on FMD infection of domestic animals will be helpful in case a new outbreak occurs and these questions are raised.
2. Initial results indicate that dromedary camels do not play an important role in the transmission of FMD virus.
3. Point estimates have been determined but the confidence intervals are wide.

Recommendations

1. A good knowledge of status (census, localisation) of native wild boars and deer is suggested. An up to date census of zoological collections, game farms and hunting properties would help assessing risk of disease spreading in case of outbreak.
2. Provided that infection and excretion occur in dromedary camels, further experiments should preferably be performed in a transmission format, otherwise the most important question with respect to the role of Old World camels in FMD transmission cannot be answered.
3. EUFMD Secretariat should collate risk assessments on the situation of camelids on holdings close to infected premises.

Item 13.

Workplan

Item 14. Any other business
NONE

Adoption of the draft report

The draft report was adopted subject to the agreed amendments being included.

Closing Remarks

The Chairman thanked members of the group for their efforts over the year and during the meeting, which had contributed to lively discussions and to a highly productive Session. He led the Session in a vote of thanks for the marvellous hospitality and organisation provided by the FLI, and in particular the personal attention given by Professor Mittenleiter and Bernd Haas to ensure an excellent venue and smooth co-ordination of all arrangements.

Appendix 1

Closed meeting of the Research group of the Standing Technical Committee of the EUFMD Commission

Friedrich Loeffler Institute, Insel Riems, Germany
20-23 September 2005

Provisional Agenda

Themes and items

Action points from the 2003-5 work plan

1. FMD risk and the priority antigens for European vaccine banks
2. Inter-laboratory laboratory proficiency tests
    a. Results of FMDV pilot study, Plans for next two years
    b. Inter-laboratory laboratory proficiency - FMD sero-diagnostic tests; status report, Phase XIX
3. Progress on surveillance post-emergency vaccination issues
    a. Progress in NSP test validation; cattle, pigs, sheep
    b. Surveillance guidelines
    c. Simulating application of PVS - scenario analysis

Raised by the 36th General Session

4. FMD control policy - progress, approaches, issues and gaps in the policy decision area.
5. FMD network – FAO/OIE epidemiology and laboratory network developments.
6. Size of antigen doses holdings in the international vaccine bank.

Other items and work plan

7. Other items from the 2003-5 work plan, or arising (list below).
8. Work plan 2005-7.

<u>Other items - the need for a report and time allocated is subject to Chairman</u>

1. Progress or discussion on status of 2003-5 work group actions
Action 2: Global FMD surveillance mapping/modelling
Action 8: Laboratory Contingency Plans –follow up to Cordoba workshop
Action 9: Guidelines for FMDV sample transport – updating issue
Action 10: European Diagnostic reagent bank
Action 11: Laboratory sero-diagnostic capacity
Action 12: Serological Laboratory biosecurity guidelines
Action 13: Pen-side tests – evaluation and guidelines
Action 15: Potency of vaccines produced in Turkey
Action 16: FMDV Inactivation kinetics – meat and milk

2. Items arising from country members/EUFMD actions

1. Biosecurity measures applicable to laboratories which intend to undertake FMDV detection tests that do not produce/use live virus (inactivated pathogen detection systems –iPADs). (Bernd Haas will provide discussion paper).
2. Risk of FMDV type Asia-1 entry into Turkey from eastern neighbours.

Provisional Timetable

Tuesday 20th		*Co-ordinator or presenter*
8.30 AM	Welcome and Introduction Meeting procedures, Election of the Chairperson Update on EUFMD Commission and ongoing/planned actions under the EUFMD Strategic Plan 2005-8 and EC-FAO agreement Summary of Research group Action Plan 2003-5	 Keith Sumption Keith Sumption Kris de Clercq
	FMD risk and the review of priority antigens	
9.30	FMD risk and the review of priority antigens for European vaccine banks [update on relevant cross-protection tests requested]	David Paton
10.10	***Break***	
	Question: Risk of FMDV type Asia-1 to Turkey	
10.30	FMD Surveillance report – risk situation in Central Asia[1]	Manzoor Hussain, FAO Regional epidemiologist
11.00	FMD Surveillance report – risk situation in I.R of Iran	Maghsoud Jamdar/Francis Geiger
11.30	Sero-monitoring for FMD infection in the Trans Caucasus	Carsten Pötzsch
12.00	Discussion. Timetable for discussion of remaining surveillance items[2] Identify reporting/working groups	
12.30	Lunch	
14.00	**Tour of Greifswald and bus to FLI**	
16.30	Reporting/working groups[3]. At FLI	
18.30	Dinner at FLI	
22:00	Return to hotel	
	If return earlier 21:00, then presentations may be made 21:00-22:00	
	Update from surveillance actions	
	FMD surveillance in Turkey – progress report from surveillance actions in Erzurum province	Tom Murray & Sinan Aktas
	FMD surveillance in Turkey – progress report from sero-surveillance in Thrace region	Sinan Aktas
Wednesday 21st	**Question: what is the demand and who can supply the needs for external quality assurance of FMD labs? in our region**	*Kris*
8.30 am	Inter-laboratory laboratory proficiency detection tests	David

1 Paper on FMD in Tajikistan may be fitted here (Erika Carlsson, FAO animal health officer Tajikistan)

2 **Monitoring situation in Turkey**

International Surveillance gaps and priorities

International networking and issues in surveillance- OIE/FAO FMD network

Other surveillance and control issues –Question of global cessation of Type C vaccination

3 To be decided by Chairman and will relate to need for specific reports

for example

R/WG on Asia-1 risk

R/WG on international surveillance gaps

R/WG on the needs of European NRLs /experts/labs within a global FMD network.

	Reports relating to progress of work groups - *virus detection tests (pilot study)* - *-sero-diagnostic action (Phase XIX)*	
9.15	Needs for EQA support - laboratories in regions neighbouring to EU25/27	Georgi Georgiev
9.30	Discussion/plan reporting group work	
10.00	Break	
10.30	**Progress on technical questions relating to surveillance post-emergency vaccination**	Kris
10.40	Current requirements of the OIE on surveillance post emergency vaccination and issues being addressed by OIE ad hoc groups	OIE representative
11.00	*Progress report on NSP test validation – and gaps remaining*	Kris
11.20	Position paper on *Post-vaccination surveillance guidelines and their application*	David (Aldo, Matthias, Kris)
11.50	Alternative view - application of NSP tests	Aldo Dekker
12.10	Discussion	
12.30	Break	
	New methods and tools to assist application of NSP tests in sampling schemes	
14.00	Designing post vaccination surveillance – a tool box and simulation approach	Matthias Greiner
14.20	Use of Likelihoods ratios in analysis of NSP test results	Dónal Sammin
14.40	NSP Test performance – analysis using non gold standard methods	Aldo Dekker
	Improving sero-monitoring of routine vaccination programmes	
15.00	Trans-Caucasus sero-monitoring NSP analysis Trans-Caucasus sero-monitoring vaccination - SP analysis	Matthias Greiner Emiliana Brocchi
15.40	Break	
	Developments in FMD control decision support tools	
16.00	Decision support tools –Whats new? What is relevant? Where are the gaps?	Mark Bronsvoort
16.30	Discussion	
	Agenda priority setting for remainder of session	
17:18.30	Reporting/working groups	
19.00	Dinner	
Thursday 22nd	**Laboratory Bio-security and virus inactivation questions**	Kris
8.30	Biosecurity for inactivated pathogen detection (iPAD) labs	Bernd Haas
9.00	Virus inactivation studies – progress Virus survival – milk	Soren Alexandersen Matthias
10.00	Break	
	Risk assessment/management Papers for review	
10.30	FMD management in wildlife	François Moutou
11.30	Risk assessment – pork from vaccinated animals	Aldo Dekker
12.30 -45	Finish discussions. Set R/WG tasks	
PM & evening	Excursion	

Friday 23rd		
8.30-10.30 11.00	Reporting/Group work - continued Work Plan 2005-7	Kris
12.00	Any unfinished business	
	Open Session 2006	
12.30	Break	
14:00	Any other business	
15.00	Break	
16:00	Report presentation	
17.00	Close	

Appendix 2

REPORT ON THE COMMISSION'S ACTIVITIES IN 2003 AND 2004

Keith Sumption

Key Points

1. The activities have followed the recommendations of the 35th Session and can be grouped under four categories:
    i. actions to prevent entry of infection into south-eastern Europe through Thrace region of Turkey, and of infection or exotic virus types into Turkey and the CIS via the south Caucasus;
    ii. actions supporting risk assessment and risk management, mainly through support to countries in high risk areas to submit samples for virus typing;
    iii. actions to address technical constraints to implementing FMD control policy in member countries, mainly through EUFMD Research Group sessions and workshops and supportive technical contracts, and via work on international standards (OIE), with most emphasis on the issues relating to vaccination to live policies;
    iv. actions aimed at improving capacity to respond to FMD emergencies, mainly concerned with diagnostic laboratory contingency planning, capacity and proficiency/standardisation.
2. The Secretariat (Secretary and Clerk), funded by members contributions, have acted to implement decisions of the 35th Session and subsequent Executive Committees. In the period 2003-2004 the Commission has been greatly assisted by an additional Associate Professional Officer (supported by Ireland). Since staff costs are met by the Commission, almost 100% of the external funding (EC and FAO) goes to cover the additional costs of the actions - for example in supply of vaccines, diagnostic kits, workshop costs.
3. Under the Implementing Agreement for 200-4 for financial support from EC for activities of the EUFMD Commission, the staff of the EUFMD Secretariat provided the project management and technical backstopping through two professional officers. These officers are greatly assisted by the Chairman of the Research Group and the other elected technical experts from member countries who provide technical advice, and assist in missions usually without charge, and through contract services with the specialist institutions such as the FAO World Reference Laboratory in the UK. The position of the Commission in relation to the FAO Animal Health Service ensures that activities are co-ordinated and informed by FAO involvement in the wider region, and complement and contribute to goals and outputs of the FAO Regular Programme.
4. The majority of the external funding has been used in country in affected or high risk country situations (Thrace region, and south Caucasus) from two sources, from DG-SANCO of the EC via the EUFMD/FAO Implementing Agreement, being US$1,536,360 in 2003 and 2004[1], and from FAO resources for Technical Cooperation Projects (TCPs, circa US$ 600,000), where the EUFMD Secretary acts as lead Technical Officer[2].
5. Over half (30) of the 59 recommendations have been implemented through activities or actions, and a further 20 can be considered collaborative work in progress with the lead being taken by other organisations.

[1] Financial statements approved by the 69th and 70th Sessions

[2] The FAO projects relating to FMD in Syria and Iraq in this period were serviced by other FAO Animal Health Service officers.

6. In line with recommendations at the 35th Session, the activities have been co-ordinated with partner organisations (OIE, EC, EFSA) to avoid overlap and make the best use of specialist technical experience in the Secretariat and Standing Technical Committee (Research Group) –
    a. Co-ordination of international actions to improve risk assessment for antigen and vaccine banks, working with FAO-WRL and the OIE (ad hoc group on antigen and vaccine banks, 6/2004).
    b. Partnership in research co-ordination, with WRL/EC/OIE in the development of the Co-ordination Action for FMD and CSF laboratories (CA-FMD CSF, to be implemented in 2005).
    c. Specialised technical support to EFSA in the panel on FMD risk to Europe – assessment and management options (10/2004).

7. The major depreciation of the dollar against the euro has had serious financial consequences as the majority of costs have to be met in euro. The impact on the balance of the Trust Fund has been softened by payment of contributions which were in arrears. The financial situation reduced the Executive's flexibility to use EUFMD resources to respond to situations and to implement recommendations.
8. A longer term strategy paper has been developed following recommendation of the Executive, for the activities in the period 2005-8, to be presented at the 36th Session.
9. A revised Agreement between FAO and EC for financing of activities of the EUFMD Commission has been prepared to follow the Agreement signed in 2001 for the period to 31/12/2004. Interim measures to finance activities have been necessary in the period before the new agreement is in place. This aspect highlights the importance of a sufficient and independent financial base for EUFMD actions.

Actions to prevent entry of infection

10. The situation of risk to EUFMD member countries was kept under continual review through the mechanism of Tripartite group meetings and at the 3 meetings of the Executive Committee, resulting in decisions to continue or implement support actions in Thrace region and south Caucasus in the period:
    a. supply vaccine for Thrace in 2003 and sero-surveillance activities in 2004;
    b. support buffer zone vaccination/surveillance support in the Caucasus in 2004;
    c. support field surveillance exercises in Anatolia (Turkey) and formulation of studies to better monitoring of FMD risk situation in eastern Anatolia.
11. The EUFMD actions, under the FAO/EC/OIE Tripartite, have assisted to stabilise the FMD situation at these border regions for Europe. However, the surveillance activities supported by the Commission in Thrace region and the south Caucasus indicate that FMDV entry occurred in the period, but without significant extension in involving neighbouring countries free of infection. The effective use of FMD vaccine in small and large ruminants in Thrace region of Turkey in this period undoubtedly contributed to control the spread of infection.
12. With FAO TCP support, a set of surveillance actions for the early detection of transboundary disease risk (FMD, PPR, sheep and goat pox and bluetongue) were introduced in 2004 into Thrace region of Turkey, complementing actions in Greece and Bulgaria; this action assisted the detection of PPR infection in Thrace and contributed to prevention of entry into Greece and Bulgaria. The westward movement of PPR across Turkey into Thrace highlights the value of the Commission tracking other diseases as indicators of animal movement, and thereby FMD risk.
13. The situation of FMD virus circulation in other risk areas was kept under continual review through the mechanism of the Executive Committee and the Standing Technical Committee meetings. FMDV surveillance information for much of the endemic areas of the world remains in critically short supply, and following recommendations arising from a review by the Standing Technical Committee, the Executive decided to support

small projects to address gaps in information of suitability of the European vaccine banks, especially to counter risk from virus types in the Horn of Africa.

Actions supporting risk assessment and risk management

14. Activities to note in the period 2003-4 have been:
    a. New actions (small projects) to support virus identification and typing in epidemiologic regions considered to pose a significant risk, and thereby to determine the suitability of antigens held in the European vaccine banks holding exists;
    b. New activity, in support of FMD risk assessment by EFSA, mainly through work on models for better identification of FMD distribution and prevalence (10/2004);
    c. Continued reporting to the Executive on changes in patterns of FMD circulation and risk to EUFMD member countries;
    d. Continue support to the FAO WRL, financially and operationally, from the EUFMD Trust Fund;
    e. From the above, it is clear that the need to address the deficiencies in data on both FMD incidence is of high importance for several functions of the Commission. The lack of systematic data on human and financial resources available to veterinary services in endemically affected countries affects identification of policy options for intervention in third countries. The international organisations could be assisted by the EUFMD Commission as a specialised body, to bring FMD information and veterinary resource information together to assist identification of effective policies for countries at different levels of development.

Actions to address technical constraints to implementing FMD control policy

15. In line with recommendations of the 35th Session, activities to address technical issues have increased significantly:
    a. Supporting the implementation of a Research Group workplan developed in 2003;
    b. Supporting resolution of vaccination to live issues, specifically
        i. gaps in NSP test validation through project grants to obtain suitable sera (Israel, Zimbabwe, Hong Kong SAR)
        ii. workshop for NSP test comparison and validation in 2004
        iii. working group on surveillance after emergency vaccination in FMD free countries
    c. Co-ordination of the efforts to increase efficiency of transfer of technical progress into practise, including revision of standards, through:
        i. setting up and steering of a Coordination Action project (with WRL and OIE) to begin in 2005;
        ii. technical advisory inputs of the Secretary and Chairman of the RG to OIE ad hoc groups, the European Food safety Authority (EFSA) panel on FMD, and as as FAO representative to the European Technical Platform for Global Animal Health.
16. The STC met on two occasions and continued its position as the pre-eminent, international forum for review of progress in FMD diagnostics and vaccination issues, with a record attendance of observers at the Open section of the Session in 2004.

Actions aimed at improving capacity to respond to FMD emergencies

17. Activities to support capacity in European countries to control FMD emergencies:
    a. Workshop for EUFMD member countries on contingency planning for FMD diagnostic laboratories attended by delegates from almost all member countries;

b. Addressing issue of lack of diagnostic capacity for post-outbreak surveillance, through development of bio-security standards that assist rapid licensing of regional/decentralised facilities;
c. Updating and continual professional development through wide participation (a record level) of European countries, and from Mediterranean basin, in the Open Session of the Standing Technical Committee, 2004.

Special Events

i. The 50th Anniversary of the foundation of the EUFMD Commission was commemorated in June 2004, through an evening hosted by the Irish Government and attended by representatives of most of the 33 member countries and each of the 6 founding member countries. Commemorative awards were made to 25 individuals whose efforts were seen as outstanding in the control of FMD in Europe and to five institutes to recognise their special contribution to the success.

Member countries

18. The number of member countries remained at 33 of which, since May 2004, 22 are EU members. No new member countries joined the Commission in the 2003-2004 period. The Republic of Ukraine officially declared interest to join the Commission in this period[3].

1. General Situation

The general situation of FMD in the world in 2005 appears similar to that of the early-mid 1990's; a relative calm, with few major incidents in previously free countries. As in the 1990's, the relative lack of headline forming incidents is most likely a very poor guide to true level of virus circulation within many endemically affected countries. Incidents of trans-boundary spread of infection were recorded in this period from South America, sub-saharan Africa, in west, central, south and south-east Asia. Extension of distribution of virus types/topotypes was recorded in this period in South-East Asia, indicating that disease ecology is not stable, and that borders remain relatively porous in several regions of the world.

However, many of these trans-boundary events did not make headline news since they involved countries not officially free of FMD.

In the absence of quantitative risk assessments, there is no reason to consider there is a reduction in risk to Europe in 2005 compared to 2003.

The available official information reported to the OIE on FMDV circulation in most endemic countries is currently so scarce, or for some countries not given at all, that it greatly constrains quantitative risk assessment. This restricts assessment of both risk of virus entry through illegal and legal routes of entry.

Following the 35th Session the Commission has:

- Contributed to the FMD risk assessment work of the European Food Safety Authority (EFSA), particularly addressing the issue of estimated prevalence of infection in potential source countries;
- In support of risk assessment, supported a pilot study aimed at improving identification of distribution and intensity of infection in risk areas (FMD Homelands pilot study);
- Initiated small projects to improve virus submission to the WRL from risk areas where information was considered most deficient;

[3] The Republic of Georgia submitted its article of acceptance of the Constitution in March 2005. The dates of entry into membership will be given in the official response of the Director General of the FAO.

- Supported State sector institutions to collaborate with European laboratories to investigate FMD epidemiology in areas where vaccination is performed (Turkey, Israel, Zimbabwe and Hong Kong SAR).

Given that Turkey and Israel are members of the Commission and that FMD was reported in each country in the period 2003-2004, the risk situation of most immediate concern is that of countries neighbouring to Turkey and Israel.
Of these, the FMD situation in Iran is of most significance given the importance of the country over the past 10 years, and longer, in the transit of FMD from south Asia to the Balkans, and via the trans-Caucasus to Turkey and Russian Federation.
In 2003 the type A-Iran-99 topotype was re-isolated in eastern Turkey after a period of several years' apparent absence, suggesting a re-entry of the topotype from neighbouring countries. Although the type A vaccine used in Turkey provided a measure of control in experimental studies, the spread of the topotype provides a significant reminder of the need to address deficiencies in vaccine application in at risk areas of Turkey, and also the need for earlier warning of movement of virus variants.
In the context of changing policies towards use of vaccination in the emergency response, early detection of antigenic variants from potential medium-high risk source countries is critical. In this respect the threat to Europe of the circulation of type A viruses of Iran-86 antigenic type, against which the type A vaccine used in Turkey is poorly protective is of great concern. For this and other reasons, the situation in Turkey and in Iran and the Caucasus is currently of primary concern for EUFMD.

FMD in the European region
The last reported outbreak ("case") in the devastating type O epidemic in Europe in 2001 occurred on 30/9/01, in England, and the United Kingdom regained its FMD free status with the OIE on 22 January 2002, and the EU member states have been FMD free following this.
The Republic of Turkey reported 76 FMDV outbreaks in 2004, up from 51 in 2003, 48 in 2002, and closer to the level reported in the previous years (88 in 2001 and 110 in 2000). Type Asia-1 was not reported after 4/2002, but of great concern is the pattern of type A infection, where the Iran-99 topotype re-appeared in 2003, and following the upsurge in type A in 2002.
EUFMD with EC support have supplied trivalent vaccine for immediate use in large and small ruminants in Thrace region in spring 2003, and thereafter the stratagy has been to support sero-monitoring of vaccine performance and of virus circulation.
FMD outbreaks have not been reported in Thrace region since 6/2001, and sero-surveillance activities supported by EUFMD/EC indicated that the strategy of twice yearly vaccination in cattle, and once yearly in small ruminants, together with other controls, has succeeded to prevent significant extension of infection entering in this period. The risk of continued incursions of virus was highlighted by the incursion of PPR infection into Thrace for the first recorded time in 2004.
The temporal and-spatial distribution in Anatolia was assessed in a collaborative study by FAO with Government of Turkey and although different trends were detected for types A, O and Asia-1, particular Provinces appeared highly important in the persistence of infection. The nature of animal production and marketing patterns supports high level of seasonal mixing and movement of animals, and may underlie the observation that of hotspots for FMD occurrence. A mission to one of these areas in eastern Anatolia in 9/2004 indicated a high importance to better identify the pattern of local and distant virus transmission in order to define feasible control options in areas affected by multiple virus types.
FAO has introduced GPS devices and GIS system (mapping of circa 40,000 epidemiological units) to the GDPC for outbreak investigation which has been used in Thrace region and in Anatolia.

The **Republics of Georgia, Armenia and Azerbaijan** did not report confirmed cases of FMD in 2003 and 2004 to the OIE. Georgia reported a suspected case in 2004 but this was not

confirmed by ARRIAH from the samples submitted. These countries remain at a high level of risk given the level of reported outbreaks in the bordering Provinces of Iran and the proximity to "hot-spots" of infection in Turkey, as well as the risk of virus entry through activities relating to internal security problems.
Sero-surveillance actions commissioned by EUFMD and undertaken by FGI-ARRIAH (Vladimir) in mid-2003 indicated high levels of recovered animals on both sides of the border between Georgia and Armenia. The results suggested but did not confirm virus circulation since the 2002 outbreaks in this region.

The situation in **Iran**, with over 1000 outbreaks in 2003 and over 600 in 2002 and over 1000 outbreaks in 2001, of types A, O and Asia-1, remains of great concern to the EUFMD Commission; this is one of the highest annual incidence rates of recorded FMD in the world (> 3 cases /1,000 head of cattle per year in last 5 years, and approximately half this in small ruminants). Distribution of infection is also very widespread, and temporo-spatial analyses by FAO indicate an even higher significance of the internal animal movement than is the case in Turkey.
Iran had until 2004 an excellent record of FMD reporting on a monthly basis to OIE, but since this period data by Province and new outbreaks are not given on a monthly basis. These problems highlight the importance of implementing the project proposed in 2003.
The potential spread of Asia-1 outbreaks is of particular concern to the Commission. Enquiries of the Secretariat indicated outbreaks were mainly restricted to the two most eastern Provinces. This information is in line with other findings from central Asia, where Asia-1 was confirmed as having spread to Tajikistan in 2003 (probably from Pakistan via Afghanistan). Unconfirmed reports of FMD occurring in 2003 in Uzbekistan, Kirghizstan and Kazakhstan were also received, and may represent an epidemic extension during 2003 from the reservoir of Asia-1 to the south.
The situation in the central Asian republics is of concern, principally the unclear status of countries with land borders to Afghanistan, Iran and China, and the risk of spread to the Russian Federation.
However, given that the entry to Europe via Iran/Turkey in to the Balkans has recently occurred, the situation in Iran, where a diverse antigenic range of FMD viruses are circulating, has immediate priority for EUFMD member countries. The Iranian veterinary authorities continued to press for a collaborative project with the Commission under EC and it is disappointing to report that the funds requested from the EC to support improved surveillance, made following the 35th Session had not been released by the end of 2004.
In the interim period the Commission supported interaction between the IVO surveillance specialists from the Iranian veterinary organisation, for example to present information to the Open Session of the RESEARCH GROUP in Crete.

In Syria and Iraq, neighbouring countries to Turkey, FAO has continued to be involved in support to animal health services in 2003 and 2004, despite the very significant obstacles. The FAO TCP support to Syria (TCP/SYR/2908) which concluded in 2/2005 has included technical support to establish GLP in FMD laboratory diagnostic procedures. The terminal statement of the TCP recommended measures to address a lack of sample collection in the passive surveillance system. In Iraq, support has broadened to include the restoration of veterinary services through the Government in Baghdad (OSRO/IRQ/406/UDG, implemented 10/2004) in extension to the support given to the three northern Governorates. In both countries surveillance remains very weak and FMD can be considered endemic, and is apparently given relatively low priority by central Government. This poses an obvious challenge for effective intervention.

2. Implementation of the 35th Session recommendations

1. In contrast to the 34th Session, the majority (30) of the 57 recommendations of the 35th Session were directed to the incoming Executive Committee for implementation in the

biennium 2003-4. In the majority of the other recommendations, partner organisations or national veterinary services were expected to respond and the EUFMD Commission through the Secretariat or elected members of committees to support the efforts.

2. As a consequence of above requirements and to efficiently co-ordinate efforts with partners, the Secretariat and member of the Research Group have committed greater amounts of time to work in committee (OIE, EFSA, EUFMD ad hoc and working groups) to address issues of common concern.
3. A table is attached indicating the reports of progress for each recommendation. Of the 59 "action points", there is evidence for about half (30) of these having been undertaken, about one-third (20) have been implemented in the period and are ongoing actions (interim reports of progress) or have implemented only in part, and for the remaining (9), actions have not been undertaken for a variety of reasons.
4. Where the recommendation was directed at member countries, the EUFMD Commission has in some cases (laboratory contingency plans, R30, and marketing authorisations for emergency vaccines, R39) sought to verify if follow up actions have occurred, through a workshop for the former and a questionnaire survey for the latter.
5. Regarding the recommendations for EUFMD activities where the recommendation has not been implemented:
    a. R7 - FMD research inventory. This was first envisaged as a survey to be conducted by the Secretariat, but since there appeared a good opportunity to gain funding for a more extended survey from DG-Research, an ERA-NET proposal (Coordinated by the Chairman of the Research Group) was developed and submitted, unsuccessfully to DG-Research. As a result the research inventory was included as a work package in Co-ordination Action for FMD and CSF laboratories, (Co-ordinator: David Paton, FAO-WRL, Pirbright) which gained approval from DG-Research and will begin in April 2005. The recently launched European Technology Platform for Global Animal Health has also identified the need for such an inventory.
    b. R33 - Annual review of Diagnostic Activity. This was discussed at the 2003 Research Group meeting and it was recommended that this should occur every two years and, following designation of the Community Reference Laboratory (CRL-FMD) for FMD, it was expected this would become an activity of the CRL.
    c. R35 - Survey of serological preparedness. As R33, it was expected this would be reviewed after 2 years at the 2005 Research Group meeting, or taken over by the CRL and reporting to both responsible EC Directorate and to the EUFMD Executive.
    d. R40 - Recommendation is for member countries, and progress should be revealed by the 2005 EUFMD survey.
    e. R44 -Guidelines relating to intentional FMDV introduction (agro-terrorism). No specific action has been taken on this.
    f. R48 - To Research Group – considered in 2003 but not prioritised for action.
    g. R52 - Workshop on QA/QC of FMD diagnostic laboratory testing; not prioritised for action at 2003 Research Group Session.
6. Recommendations from the 34th Session

It should be noted some recommendations of the 2001 (34th) Session had not been acted upon by 2003, but have been in the period since then.

In particular:

- Recommendation 6.4, 34th Session; the Commission's activities in risk assessment have been pronounced working to support import risk analysis in support of EFSA panel on FMD risk.
- Recommendation 1.10, 34th Session; relating to contingency planning for FMD diagnostic capacity for crisis situations, a workshop was organised in April 2003,

attended by around 40 participants from across Europe, with papers developed by Research Group and reviewed as part of the workshop.

However, the Secretariat draws attention to Recommendation 9.2, 34th Session; that the Commission prepares guidelines on the correct protocols for the transport, handling and administration of emergency FMD vaccines has not been specifically addressed by the 35th Session, or by the 36th Session. The 36th Session or the Executive could usefully decide on the value of producing these guidelines. These guidelines could be extended to address the issue of planning for mass deployment of vaccination under timescales expected of emergency campaigns.

3. Specific Activities

1. The **Executive Committee** held three ordinary Sessions, the 69th in Ohrid, FYR of Macedonia, 23 & 24 October 2003, with follow up meeting in Rome on 1st December 2003, the 70th in Dublin, 9-10th June 2004, and the 71st in Rome, 23-24th January 2005. The Reports of the 69th and 70th Sessions, in English and French, were sent to all member countries and are available on the EUFMD website, and the 71st is planned for circulation by the end of March 2005.
2. The **Research Group** of the Standing Technical Committee of the Commission held two Sessions during the biennium; one with restricted participation, held at Gerzensee, Switzerland, 16-19th September 2003, attended by 28 technical experts, and a Session with an Open Section for observers in 2004 in Chania, Crete, 11-15 October 2005, attended by over 130 FMD specialists. The latter is a record attendance at Research Group Session, from almost all of the National FMD laboratories in Europe including the ARRIAH OIE Regional Reference Laboratory, and in addition representatives from FMD reference Laboratories in the Americas, in Africa, and from national reference laboratories in the Middle East and north Africa, Iran, Ethiopia and Kenya, and from India, Hong Kong and Australasia. This again highlights the importance of these meetings for stimulating technical improvements and capacity building in EUFMD member states and in neighbouring (mainly infected) countries in the region. The reports of the two sessions were sent to all European FMD Research Institutes and laboratories and were posted on the EUFMD website. The Chairman of the Research Group was also actively involved in the activities of the Commission, representing the Research Group at internal and external meetings.

3. Three **workshops** were held in the 2003-2004 biennium:
   A workshop on **FMD outbreak investigation** principles and procedures was held in English and Russian in FAO offices in Budapest, March 2004, for senior state officers from western Balkans and CIS countries (three Caucasus countries, Moldova, Russian federation, Tajikistan and Ukraine).
   A joint EUFMD/EC workshop on **FMD Contingency Planning** for FMD Laboratories was held between the 28-30th April 2004, in Cordoba, Spain with 40 participants from 32 countries, including 21 of the 25 EU member states. The report and recommendations were published in the 70th Session report.
   A workshop to **evaluate NSP tests for use in Europe** was held in Brescia, 18-22 March 2004, funded by EUFMD and EC (DG-SANCO and DG-Research/ImproCon), to undertake comparison of OIE reference test, commercially available and in-house assays for detection of antibodies to NSP antigens using panels of bovine and other sera supplied by the participants. Participants were from 8 European NRLs and the PAHO Panaftosa Laboratory.

4, The EUFMD/EC/OIE Tripartite Group for FMD control in the Balkan region

The Group met on 10th October 2003 in Ankara, Turkey, and in November 2005.
The 2003 meeting was important for the:

- review of the information on the EC supplied vaccine in spring 2003 campaign, and the progress of the autumn campaign in Turkish Thrace;
- review of the EUFMD sero-surveillance plan for monitoring of vaccination/presence of recovered animals, implemented by the GDPC in summer 2003;
- Review of use of kits and equipment supplied in 2003 to enable sero-monitoring.

Review implementation of EUFMD expert mission on vaccine quality assurance in June 2003, which had been necessitated by the poor performance in external potency tests of vaccine produced in the SAP Institute.

Arising from the Tripartite group, the 3 countries had in 2003, for the first time as a joint action, proposed a regional Technical Cooperation Project (TCP) to FAO on FMD and other exotic disease surveillance in Thrace Region. The TCP was implemented with the Secretary, EUFMD as lead technical officer, and commenced in October 2003.

The 2004 meeting was important to review and monitor:

- The vaccination campaigns in 2003/4, and especially the serological levels of protection following use of FMD vaccine produced in spring 2004 in Turkey;
- The sero-monitoring for virus circulation, and subsequent investigation of reactors;
- The mission to eastern Anatolia to test draft outbreak investigation procedures/guidelines;
- The PPR situation in Thrace region, and particularly the reports of the EUFMD/FAO emergency mission in October 2004;
- Emergency reporting arrangements for information to neighbouring countries of new disease events;
- Proposed actions for FMD control in Anatolia;
- Regional collaboration in early detection of Blue tongue risk.

As a result the Turkish authorities agreed to develop an emergency action plan for PPR in Thrace region, with creation of a local disease control centre (LDCC) and with the strategy of PPRV eradication from Thrace, with additional measures to prevent re-introduction.
A sero-monitoring plan for 2004 was developed for Thrace with a focus on FMD, which was agreed by the GDPC and has been implemented in early 2005.
The actions described are in line with the principles of the **longer term strategy for FMD control in Turkey** which was agreed by the FAO/OIE/EC Tripartite at the 25th October 2002 meeting, for progressive control that could establish disease free zones in Turkey by 2008, and "the aim for 2008 would be that FMD is no longer endemic in the region, and that epidemics as a result of trans-boundary spread are limited in number, occur only in identified high risk zones and can be rapidly eliminated without extension out of these zones". It is clear there will be a need to monitor progress whether or not EUFMD is required to provide technical or financial support.

5. The OIE/EUFMD-FAO/EC Tripartite Group for FMD control in the Trans-Caucasus countries

The group met on the following occasions:
1st November 2004, in Kiev; 15th March, in Budapest, to review the technical reports of the 2003 buffer zone vaccination, and in May 2004 in Paris.
Following the 1st November meeting, training was provided for laboratory staff from the three countries in early 2004. The meeting in March did not support further vaccination in buffer zone until indications were clearly received that FMD cases would be immediately reported to the OIE and that samples would be submitted in timely manner for virus and vaccine matching; in May 2005 it was decided to reconvene buffer zone vaccination with support from EC through EUFMD, for autumn 2004 and spring 2005.
FAO would at the same time provide a TCP project to assist capacity building in surveillance, and to assist the countries to develop contingency plans. The meeting emphasised the importance of development of a longer term project to follow interim actions in 2004/5, with EUFMD as co-ordinator under the EUFMD/OIE/EC Tripartite. The 70th Executive

Committee modified the draft proposal, and supported immediate efforts to put in place a Regional Technical Co-ordinator. As an interim measure because of financial restrictions, a co-ordinator was recruited for two missions in 2004/2005 (Dr Carsten Pötzsch (FLI, Germany).

6. Activities relating to FMD control in Iran and Central Asia

The exceptional importance of this region as a source of FMDV antigenic variants for Turkey and neighbouring region is well recognised. For example, Asia-1 swept through Iran in 1999 into Turkey and travelled as far as Greece in 2000, and in 2003 Asia-1 extended its distribution into Tajikistan, and unofficial reports indicate into neighbouring countries in central Asia.

In 2003 the EUFMD Commission, following the 35th Session recommendations, made a request for financial support (761,000 US$ in Phase 1) from the EC for a programme of actions aimed at strengthening surveillance in Iran, especially in areas of most concern for early detection of FMDV threats to Turkey. In the subsequent period, it was clear that the parties concerned including the Iranian Veterinary Organisation (IVO) were fully supportive of the need for the programme but the necessary financial guarantee by the EC was not forthcoming. Strong indications were received in late 2004 that funding should be available in 2005. Given the latter, and need for action, the Government of France made good their offer to provide technical assistance to the programme through a veterinary technical officer (Dr Francis Geiger), to be based in Teheran and to work under EUFMD Commission for 3 years. The lack of funding agreement has constrained the working relations between EUFMD and the IVO, and it is clear that Dr Geiger can only work on a temporary basis with the IVO until the work programme is funded by the EC.
In order to maintain communications and relations during this period, surveillance specialists of the IVO were supported to attend EUFMD Research Group meetings (Crete, 2004).

In 2004, FAO implemented a transboundary animal disease surveillance and control project (GTFS/INT/907/ITA) for central Asian countries, with a focus on Afghanistan, funded by Italy (2.8 million US$) This project should provide significant information to assist early warning of disease movement in the region, and there is extensive opportunity for collaboration and mutual benefit with the planned EUFMD actions with Iran.

7. Collaboration with the Office International des Épizooties (OIE)

The Commission has worked closely with the OIE:

- in FMD control through the two Tripartite groups;
- in development of new initiatives in Iran/central Asia;
- Through the participation of the Secretary, and two members of the Research Group in the OIE ad hoc group vaccine and antigen banks, from June 2004 continuing into 2005;
- Through the participation of two members of the RG in the OIE ad hoc group on validation of NSP tests, in 2004;
- Through participation of FAO-WRL in the meetings of the OIE Scientific Committee on Animal Diseases (SCAD);
- The OIE has been invited as observer to all EUFMD Sessions, including Closed Sessions of the RG;
- In the development of a Co-ordination Action for FMD and CSF laboratories, co-ordinated by Dr David Paton in which the EUFMD Commission and OIE are members of the Steering Committee.

The above has enabled several of the recommendations of the 35th Session to be carried forward more expeditiously through participation to OIE ad hoc groups and other common actions.

8. Collaboration with the WRL and other national laboratories

The FAO World Reference Laboratory for FMD continues to play a very important role in the Commission's activities, with the WRL represented at each of the EUFMD Sessions and providing services through a contract with EUFMD and with the FAO regular programme and experts for workshops.

Additional contracts (Letter of Agreement) have been placed with the WRL in 2004 to undertake laboratory services in validation of NSP and other DIVA tests, relating to studies on SAT virus infections. As a condition of the contracts, the WRL was required to keep available the sera for other European laboratories involved in NSP test comparison.

The Secretariat has worked closely with WRL to implement support to NRLs in African and other countries to support delivery of samples for virus typing. The requirements of each situation are different and the process of supporting countries demanding in effort, and the close co-operation of WRL and EUFMD has been important to overcoming obstacles and building trust with the countries concerned.

The Commission also financed the FAO-WRL Phase XVII project, which continued to provide external quality assurance (proficiency test panels) to laboratories of member countries, and continued development of international reference sera - and can be considered to be the pre-eminent international initiative in FMDV diagnostic standardisation.

9. New member countries

No new country has joined in the biennium. Following the 68th Session in Vilnius, the CVOs of the Republics of Estonia, Latvia and Slovakia have been contacted to ascertain their interest in membership of the Commission. The Republic of Latvia has expressed interest but no response has been received from the other states.

The Republic of Ukraine officially indicated an interest to join the Commission in 2004; formal membership cannot begin until submission of the article of agreement to the EUFMD Constitution.

Under the current Constitution of the EUFMD, countries are eligible for membership if they are served by the FAO regional office for Europe and are either members of the FAO, or if not FAO members, they are members of both the OIE and the UN. The table below illustrates the position of non-members states of the EUFMD in the European region. The membership of other European states, whose proximity to FMD infected countries ensures that their role in FMD control will remain significant, is an open question.

Country	***FAO member***	***OIE and UN members****
Armenia	Yes	Yes
Azerbaijan	Yes	Yes
Belarus	No	Yes
Bosnia-Herzegovina	Yes	Yes
Georgia	Yes	Yes
Russian Federation	No	Yes
Moldova	Yes	Yes
Ukraine	Yes	Yes

10. Publications

Official Session reports and Workshop Proceedings are given in the Annex to this report.

11. Missions

Details of mission travel in 2003 and 2004 are given in the Annex to this report.

PUBLICATIONS – 2003/2004

Title	*No. of pages*	*No. of annexes*	*Language*		*Cost of re-prod.* *	*Cost of trans.*
			Eng	*Fr*		
2003						
Report of the 35th Session, Rome 9-11 April 2003	192 E 205 F	18 E	x	x	Nil	US$9,000 *(report & annexes)*
Report of the Session of the Research Group, 16-19 September 2003, Gerzensee, Switzerland	169	25	x		Nil	
Report of the 69th Session of the Executive Committee, 23-24 October 2003, Ohrid, TFYR of Macedonia and Follow-up meeting, Rome, 1 December 2003	83 E 83 F	13	x	x	Nil	$1,500
2004						
Report on the Workshop on contingency planning for FMD laboratory diagnostic activities, 28-30 April 2004, Córdoba, Spain	62	10	x		Nil	
Report on the Workshop on FMD outbreak investigation, 16-19 March 2004, Budapest (Consultant report: Nicholas Taylor)	52	3	x		Nil	
Report of the 70th Session of the Executive Committee, 9-10 June 2004, Dublin, Ireland	84 E 84 F	15	x	x	Nil	$1,500
EUFMD Activities and Achievements 1954-2004	32		x	x	Nil	
Report of the Session of the Research Group, 11-15 October 2004, Chania, Crete (Greece)	491	82	x		Nil	
Book of Abstracts for the Research Group Session	75		x		Nil	
2005						
Report of the 71st Session of the Executive Committee, 24-25 January 2005 - *under preparation*						
* *Reproduction costs covered by AGA Regular Programme funds*						

DUTY TRAVEL – EUFMD SECRETARIAT

2003

DUTY TO:	*DATES:*	*PURPOSE*	*FUNDING*
KEITH SUMPTION			
Denmark UK	15-16 January 17-19 January	Denmark:Discussions with Head of International Epilab on preparation of annex on design of surveillance of FMD; UK: Invitation from Pirbright to meet new Director of institute and discuss 2003 activities	TF
Paris, France	31 January – 5 February	Mtg. on global framework for control of FMD and other TADs and annual FAO/OIE/WHO Tripartite mtg	TF
Paris, France	11-14 February	OIE FMD and other epizootics commission	TF
Brussels, Belgium	18-19 February	Visit EC to discuss EUFMD matters	TF
Strasbourg, France	16-18 March	Attend European FMD technical and policy symposium	TF
Paris, France	18-23 May	Attend 71st OIE General Session	TF
Ankara, Turkey	17-21 June	Mission on vaccine quality control and surveillance	EC TF
London, UK	25-27 June	Review DEFRA FMD research programme at FAO WRL, Pirbright	Insurance only
Anatolia, Turkey	20-30 July	Sero-surveillance mission	EC TF
Gerzensee, Switzerland	15-20 September	Session of the Research Group of the EUFMD	TF
Cairo, Egypt Ankara, Turkey	4-11 October	Cairo: Roundtable on FMD; Ankara: EUFMD/EC/OIE Tripartite on the Balkans	TF
Ohrid, FYR of Macedonia	22-26 October	69th Session of the Executive Committee of the EUFMD	TF
Kiev, Ukraine	31 October – 2 November	EUFMD Tripartite on the Caucasus	TF
DÓNAL SAMMIN			
Copenhagen, Denmark	14-21 June	Attend course on risk assessment	TF + APO funds
Anatolia, Turkey	20-30 July	Sero-surveillance mission	EC TF
Copenhagen, Denmark	9-16 August	Course on disease control and dynamics	APO funds
Gerzensee, Switzerland	14-21 September	Session of the Research Group of the EUFMD	TF
Ankara, Turkey	8-11 October	Tripartite Group meeting	TF
Ohrid, FYR of Macedonia	21-26 October	69th Session of the Executive Committee	TF
Athens, Greece	6-14 December	Workshop on active FMD surveillance	EC TF
EGIZIANA FRAGIOTTA			
Gerzensee, Switzerland	14-21 September	Session of the Research Group of the EUFMD	TF
Ohrid, FYR of Macedonia	21-26 October	69th Session of the Executive Committee	TF

NON-STAFF			
Name/country	**Dates:**	**Location/Purpose:**	**Funding:**
Kris De Clercq (Belgium)	8-14 April	Rome: 35th Session of the EUFMD	TF
Tony Garland (UK)	8-14 April	Rome: Rapporteur at the 35th Session of the EUFMD	TF
Emma Hartnett (UK)	8-9 April	Rome: 35th Session of the EUFMD	TF
Aldo Dekker (Netherlands)	17-21 June	Turkey: Mission on vaccine quality control & surveillance	EC TF
Mustafa Tufan (Turkey)	22-28 July	Anatolia: Sero-surveillance mission	TF
De Clercq (Belgium) Moutou (France) Haas (Germany) Have (Denmark) Dekker (Netherlands) Sanchez (Spain) Paton (UK) Palfi (Hungary) Yadin (Israel) Unal (Turkey) Thurmond (USA) Collins (USA) Greiner (Denmark) Van Loon (Neth.) Gil (Uruguay)	15-21 September	Research Group Members Session of the Research Group Gerzensee, Switzerland 16-19 September Invited experts (observers)	EC TF TF EC TF
Slobodan Cokrevski (Macedonia) Georgi Georgiev (Bulgaria) Mihalis Patakakis (Greece)	8-11 October	Turkey: EUFMD/EC/OIE Tripartite Group meeting	EC TF
Kris De Clercq (Belgium)	22-24 October	Ohrid, TFYR of Macedonia: 69th Session of the Executive Committee as Chairman of Research Group	TF
Slobodan Cokrevski (Macedonia)	30 Nov – 2 December	Rome, Italy: Follow-up meeting to 69th Session of the Executive Committee	TF
Mustafa Tufan (Turkey)	7-13 December	Athens, Greece: To attend workshop on active surveillance training (under TCP/RER/2903)	TF
Lilyana Polihronova (Bulgaria)	7-13 December	Athens, Greece: To attend workshop on active surveillance training (under TCP/RER/2903)	TF

2004

DUTY TO:	DATES:	PURPOSE	FUNDING
KEITH SUMPTION			
Brussels, Belgium	20-23 January	EC to discuss research projects on FMD	TF (re-imbursed by EC DG-Research)
Budapest, Hungary	14-17 March	Tripartite Caucasus	TF
Buenos Aires, Argentina	11-19 April	OIE Conference	TF
Paris, France	4-9 May	OIE meeting on FMD control (5 May)	TF
Cordoba, Spain	27 April – 1 May	Workshop on FMD contingency planning	TF
Paris + UK	4 - 9 May	OIE Meeting on FMD control	TF
Paris	23 - 28 May	72nd OIE General Session	TF
Dublin	8 – 15 June	70th Session of the Executive Committee + Anniversary event	TF
Paris	22-25 June	OIE Ad hoc group meeting	TF
Avila, Spain	27/9 – 1/10	OIE 21st Conference	TF
Berlin	5-6 October	Plan vaccine monitoring - FMD control in the Caucasus	EC TF
Chania, Crete	9-17 October	Closed and Open Session of the Research Group	TF
UK	27 October	To hold discussions between EUFMD and EFSA on EFSA mandate on FMD	EFSA
Turkey Bulgaria	30 Oct – 3 Nov 3 – 5 Nov	*Turkey:* To discuss EC projects under development; *Bulgaria:* Tripartite Group Mtg. on the Balkans	EC TF
Kiev, Ukraine	5 – 9 December ***Postponed***	Tripartite Group Mtg. – Caucasus	TF
Brussels, Belgium	12-16 December	EFSA	TF
DÓNAL SAMMIN (APO)			
Sofia, Bulgaria	10 - 12 March	Workshop on FMD laboratory diagnosis	EC TF
Pirbright, UK	24 - 26 March	Visit Pirbright Laboratory	TF
Harare, Zimbabwe Pirbright, UK	22 April - 5 May 5 - 9 May	Assist with specimen collection from convalescent cattle; UK – return with specimens and assist with cataloguing	APO - TF
Brescia	12 – 15 May	Assist with workshop on NSP tests	EC TF
Dublin	7 – 12 June	70th Session of the Executive Committee + Anniversary event	TF
Turkey	2-6 August	Backstopping TCP/RER/2903	TCP
Anatolia, Turkey	12-25 September	Pilot study implementing revised guidelines on FMD outbreak investigation	TF
Chania, Crete	9-17 October	Closed and Open Session of the Research Group	TF
Edirne, Turkey	22 – 29 October	Unscheduled: Emergency mission to Turkey – PPR outbreak	EC TF
Sofia, Bulgaria	3 – 5 November	Tripartite Group mtg. on the Balkans	EC TF
Brussels, Belgium	9-10 December	Meeting with Alf Füssel, Kris De Clercq	EC TF
Tbilisi, Georgia	12-16	Receive training by participating at	AGA RP

	December	workshop on disease surveillance	(training funds)
SIMONA SANGIOVANNI (Volunteer)			
Budapest, Hungary	14-17 March	Workshop facilitation –FMD outbreak investigation, CIS/Balkans/ Caucasus	TF
Cordoba, Spain	27 April – 1 May	Facilitate Workshop on FMD contingency planning	TF
Brescia	10 – 13 May	Support Workshop on NSP tests	TF
EGIZIANA FRAGIOTTA			
Dublin, Ireland	7-12 June	70th Executive + 50th Anniversary event	TF
Chania, Crete	9-17 October	Closed and Open Session of the Research Group (replaced by Maria Grazia Solari)	TF
NON-STAFF TRAVEL			
Name	**Dates:**	**Location/Purpose:**	**Funding:**
Armenia: M. Khachatryan L. Galstyan T. Gasparyan	 Sofia 23/2-13/3 Sofia 23/2-13/3 Sofia & Budapest 16-19/3	 *Sofia:* Training course on FMD laboratory diagnosis *Budapest*: Workshop on FMD laboratory diagnosis	 *EC TF*
Georgia: U. Rurua K. Rusidze U. Orkoshneli	 Sofia 23/2-13/3 Sofia 23/2-13/3 Sofia & Budapest 16-19/3	 *Sofia:* Training course on FMD laboratory diagnosis *Budapest*: Workshop on FMD laboratory diagnosis	 *EC TF*
Nicholas Taylor *UK*	15-20 March	**Budapest:** Workshop on FMD laboratory diagnosis for Caucasus/Balkan/CIS countries	*EC TF*
Kris De Clercq, *Belgium* Aldo Dekker, *Netherlands* Bernd Haas, *Germany* David Paton, *UK* Hagai Yadin, *Israel* Abdulnaci Bulut, *Turkey* Ingrid Bergmann, *Brazil* Viviana Malirat, *Brazil* Erika Neitzert, *Brazil*	9-15 May 12-15 May 12-15 May 12-15May 12-15 May 9-15 May 1-18 May 1-18 May 1-18 May	**Brescia, Italy** Workshop on evaluation of FMDV serodiagnostic tests required to support "vaccinate to live" policy	*EC TF*
Kris De Clercq (Belgium)	8-12 June	**Dublin, Ireland** 70th Session of the Executive Committee and 50th Anniversary Event	*TF*

Yves Cheneau (France) Yves Leforban (France) Paul Capstick (Kenya)	10-12 June 11-13 June 10-12 June	**Dublin, Ireland** 50th Anniversary Event	*TF*
Oliver McDonagh (Ireland)	12/9 – 2/10	**Anatolia,Turkey** Pilot study implementing revised guidelines on FMD outbreak investigation	*TF*
Kris De Clercq (Belgium)	28-29 June	**Paris, France** OIE ad hoc group meeting	*TF*
Research Group members: Kris De Clercq Aldo Dekker Franco De Simone Matthias Greiner Bernd Haas Francois Moutou David Paton José Sanchez Nilay Ünal Hagai Yadin *Invited experts:* Ms Kitman Dyrting (Hong Kong) – *DSA only* Jeffrey Hammond (Australia) – *Ticket only* Abraham Sangula (Kenya) – *DSA only* Abdolahi Darab (Iran) Hassan Vishteh (Iran) Esayas Gelaye (Ethiopia) Mesfin Sahile (Ethiopia) Mansoor MA Al-Qadasi (Yemen) Najib AQ Al-Hammadi (Yemen)	11-15 October	**Chania, Crete (Greece)** Closed and Open Session of the Research Group Closed Session: 10 October Open Session: 11-15 October	EC TF TF EC TF TF TF RADISCON RADISCON RADISCON RADISCON
Carsten Pötzsch Consultant (Germany)	11 October - 6 November	**Georgia, Armenia, Azerbaijan** Consultancy mission on FMD control	EC TF
John Anderson (UK) Mustafa Tufan (Turkey) – *DSA*	24-29 October	**Turkey** Emergency mission – PPR outbreak	EC TF EC TF

only Sirin G Cizmeci (Turkey) – *DSA only*			EC TF
Naci Bulut (Turkey)	9-11 December	**Brussels, Belgium** Discussions on interpretation of Thrace serosurveillance	*9-11 Dec.* EC TF
Carsten Pötzsch (Germany) - Consultant	12-18 December	**Tbilisi, Georgia** Assist in leading FAO regional workshop on serosurveillance strategies	EC TF

Appendix 3

FAO EUFMD Research Group ACTION PLAN 2003-2005

Kris De Clercq, *Chairman of the Research Group*

Action 1: Assisted delivery for samples from third countries.

Output: to collect more samples, possibly containing FMDV, from areas where little is known about and to send them to the FAO WRL. Report to the Executive Committee (Exec. Com.) Session/General Session/Research Group (RG).

Responsible: EUFMD secretariat

Others: FAO WRL

Progress: Despite the relatively slow progress made in establishing agreements, the RG strongly recommended continuation of the efforts. In 2005 RG group to review gaps in sample submissions to reference laboratories.

Timing: on schedule.

Action 2: Global FMD surveillance map/models.

Output: animal population/movement maps, maps indicating difference in animal price, FMD risk maps/global risk analysis tools.

Responsible: EUFMD secretariat

Others: FAO WRL/ Prof. Willeberg/ FAO/OIE Working group

Progress: Pilot study done/Collaboration with FAO/Interaction with EFSA/ Ongoing collaboration with M. Thurmond, Davis, US. Liaison with EU DG Research and NATO.

Timing: ongoing, some delay.

Action 3: Vaccine strain selection.

Output: advice on FMDV strain to be integrated in vaccines or antigen banks; put up a network between FAO WRL and OIE Regional Reference Laboratories.

Responsible: FAO WRL

Others: EUFMD secretariat

Progress: EU Coordinated action started January 2005; Agreement proposal for a network adopted by OIE *ad hoc* group on Ag-Vaccine banks; Paper on vaccine strain matching to be included in the FMD chapter of the OIE Manual and scientific paper planned; Network meeting and organisation of meeting with antigen-vaccine bank managers (later with manufacturers) in 2006; In 2005 RG group to review vaccine antigens.

Timing: on schedule.

Action 4: Proficiency test for FMDV detection.

Output: Proficiency panel for virus detection methods (VI, antigen ELISA, RT-PCR).

Responsible: FAO WRL

Others: Kris De Clercq

Progress: pilot study with 5 NRL done, to be analysed and reported to the RG September 2005. Scientific paper to be finished 2005. Costing for extension to other laboratories made and funding to be discussed. Organisation proposal for proficiency test in 2005/2006 is ready.

Timing: on schedule.

Action 5: Proficiency test for detection of antibodies against FMDV.
Output: Phase XVIII and plan for next phase.
Responsible: FAO WRL
Others: Kris De Clercq / Emiliana Brocchi / Aldo Dekker / Bernd Haas
Progress: Phase XVIII concluded and reported to the RG meeting 2004. Proposal for Phase XIX made and presented to Executive Committee January 2005. Proficiency panel of sera will be sent out May 2005 and results will be reported to the RG September 2005.
Timing: on schedule.

Action 6: Comparative evaluation of candidate NSP tests (to differentiate infected from vaccinated animals).
Output: Performance characteristics for different NSP tests.
Responsible: E Brocchi/ Kris De Clercq
Others: Aldo Dekker/ David Paton/ Matthias Greiner/ Donal Sammin/ Hagai Yadin
Progress: Bench workshop with comparison of 6 NSP tests including the OIE and the European index test, May 2004. International community and manufacturers invited. Preliminary results reported to EUFMD/ DG SANCO/ OIE. Follow-up studies in Zimbabwe for cattle and in Hong Kong for pigs done. Scientific papers to be finished.
Timing: some delay.

Action 7: Guidelines on post-vaccinal surveillance (PVS): Vaccine-to-live policy for Europe.
Output: Need for guidelines and between/within herd prevalence estimates in a vaccinated population.
Responsible: David Paton
Others: Kris De Clercq/ Aldo Dekker/ Matthias Greiner
Progress: Guidelines on PVS discussed and to be worked out further; Paper on small herds introduced for publication; Comments on the OIE Code chapter 3.8.7 formulated, discussed with DG SANCO and transferred to OIE. Prevalence estimates and acceptable levels of confidence for PVS to be worked out.
Timing: ongoing.

Action 8: WORKSHOP on contingency planning for National Reference Laboratories.
Output: position papers on the different aspects of contingency plans.
Responsible: EUFMD Secretariat
Others: Kris De Clercq/ José Sanchez-Viscaino
Progress: Workshop organised April 2004 in Cordoba. Attended by NRLs from all over Europe. Several working groups (WG) (see below) produced position papers. WG5 discussed the contingency plan for laboratories as presented at the General Session April 2003 (see report 2003 appendix 11). Reviewed contingency plan for laboratories from the FAO WRL transferred to the EUFMD Secretariat.
Timing: on schedule, need for follow-up.

Action 9: Guidelines for sample transport.
Output: paper to help people in sending samples to other laboratories.
Responsible: Vilmos Palfi

Others: David Paton
Progress: A paper explaining the way to send samples to the FAO WRL and indicating the transport organisations involved, was discussed at Cordoba (WG2), reviewed and adopted. The actions initialised by the OIE were considered essential and supported trough DG SANCO.
Timing: on schedule, need for yearly update.

Action 10: Diagnostic reagents bank.
Output: position paper.
Responsible: Bernd Haas
Others: Kris De Clercq
Progress: A position paper presenting also the information from manufacturers of diagnostic kits was discussed at the Cordoba workshop (WG1). The paper was presented to the Exec. Com. and DG SANCO.
Timing: on schedule, need for follow-up.

Action 11: Guidelines on laboratory sero-diagnostic capacity (scaling-up).
Output: position paper.
Responsible: WG3
Others: Lorena Jemeršic, Diane Clery, Emiliana Brocchi, Dita Krastina, Ivan Holko, Naci Bulut, Karl Johan Sørensen, Aldo Dekker
Progress: A position paper was produced during the Cordoba workshop (WG3).
Timing: delay, need for follow-up.

Action 12: Biosecurity of laboratories involved in sero-diagnosis.
Output: position paper.
Responsible: EUFMD Secretariat
Others: WG4
Progress: A position paper was produced during the Cordoba workshop (WG4), adopted by the RG, presented to the Exec. Com. and transferred to the OIE ad hoc group on biosecurity. Translation of the paper is ongoing. Paper will be transferred to the Member Countries.
Timing: on schedule.

Action 13: Evaluation of pen-side tests and development of guidelines.
Output: performance characteristics of pen-side tests and guidelines on who could use these tests and the follow-up of the outcome.
Responsible: Nilay Unal
Others: Hagai Yadin / Donal Sammin
Progress: Study on disease outbreak investigation in Turkey with the use of pen-side test. Results presented to the Exec. Com. January 2005. Need for further analysis and investigation.
Timing: delay.

Action 14: Surveillance in endemic areas and buffer zones.
Output: guidelines on and follow-up of surveillance.
Responsible: EUFMD Secretariat

Others: Donal Sammin, David Paton, Kris De Clercq, Naci Bulut, Sinan Aktas
Progress: Analysis and interpretation of village level data of 2004 (Thrace, Turkey) discussed and follow up plan for 2005 established (December 2004). Discussion on surveillance plan for Anatolia is ongoing.
Timing: on schedule, need for follow-up.

Action 15: Potency test evaluation.
Output: follow-up of vaccines used in vaccine campaigns in Turkey.
Responsible: Nilay Unal
Others: Aldo Dekker
Progress: Results of vaccine use in the field discussed. In detail study of potency tests necessary.
Timing: delay.

Action 16: Study FMDV inactivation kinetics in animal products.
Output: Assessment of D-values and Z-values for heat treatment of milk and pork from FMD-infected animals.
Responsible: Soren Alexanderson
Others: Prof. Willeberg/ Aldo Dekker
Progress: A risk assessment study was performed by EpiLab (Denmark) to inform the experimental group about specific research objectives from a risk assessment point of view. A project proposal for pork products has to be drafted including participation of the pork industry. An initiative for milk products is almost impossible without research support and/or support from the milk-products industry.
Timing: delay.

Action 17: Information management.
Output: Establishing tools for information gathering, dissemination and use for training.
Responsible: EUFMD Secretariat
Others: Kris De Clercq
Progress: A working group has met with AVIS (private company) and worked out a collaboration (March, 2005).
Timing: on schedule, ongoing.

Action 18: Research group meetings.
Output: bringing together the FMD scientific community from the all over the world.
Responsible: EUFMD Secretariat
Others: Kris De Clercq / Hagai Yadin
Progress: A closed session is planned at the island of Riems, Germany, 20-23 September 2005. An open meeting is planned in Israel, 17-20 October 2006.
Timing: on schedule.

FMD WRL REPORT

FMD FAO World Reference Laboratory for FMD

David Paton, Nigel Ferris, Jean-Francois Valarcher, Nick Knowles, Caroline Wright, Bob Statham

Summary of key events

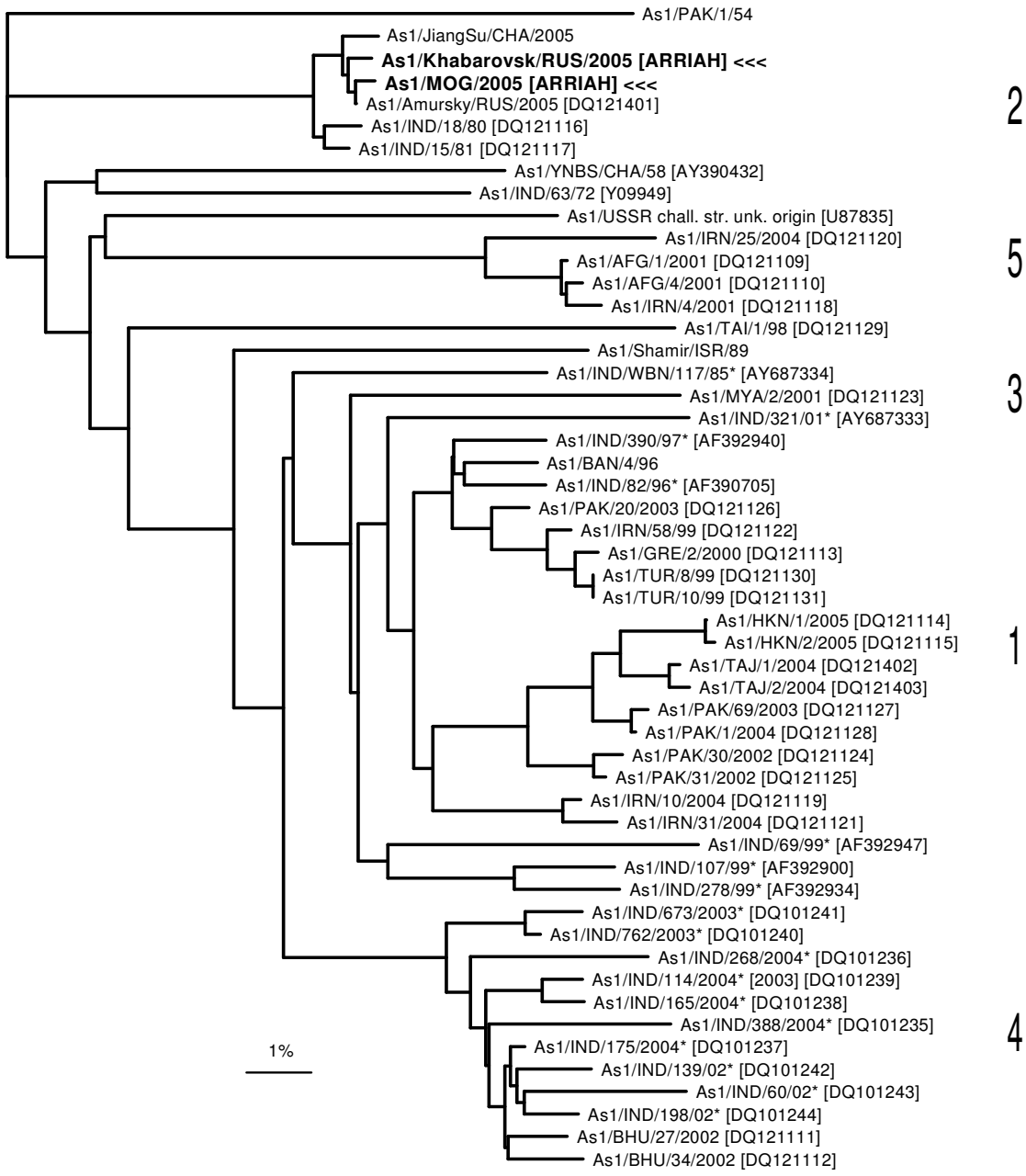
- No extensions into officially FMD-free countries that do not practise vaccination
- Asia 1 epidemic
- New free zones with vaccination in S America
- Increased vaccine efforts in India and China
- Vaccine-type viruses in East Africa
- Eastward extension of type A in SE Asia

FMD Free and not-free Countries

Asia 1 Epidemics

- At least 5 clusters of virus types
  1. Pakistan (02-04), Iran (04), Tajikistan (04), Afghanistan (04), Hong Kong (05),
  2. China (05), Russia (05), Mongolia (05), India (80)
  3. Myanmar (05), Thailand (98)
  4. India (02-04)
  5. Afghanistan (01), Iran (01-04)
- Asia 1 Shamir vaccine still appropriate?

Report on Asia 1 Russia and Mongolia 2005 sequences received from FGI ARRIAH on 28/08/2005



Unrooted Neighbor-joining tree based on a comparison of the complete VP1 gene (~633 nt). The tree was outgroup-rooted using PAK/1/54.

* Not a WRLFMD reference number.

N.J. Knowles, R.J. Midgley & J.-F. Valarcher, 28 August 2005

Samples received at FAO WRL

	2004		2005 (Jan-Aug)	
	Countries	Samples	Countries	Samples
Africa	12	335	9	109
Asia	3	72	2	35
SE Asia	3	31	5	29
Middle Eas	3	28	2	44
America	0	0	0	0
Europe	2	24	1	4
TOTAL	22	453	19	221

Serotypes detected at FAO WRL

	2004	2005
O	132	52
A	7	19
C	0	1
SAT 1	30	7
SAT 2	46	15
SAT 3	0	0
Asia 1	3	11
SVD	24	0
TOTAL	242	105

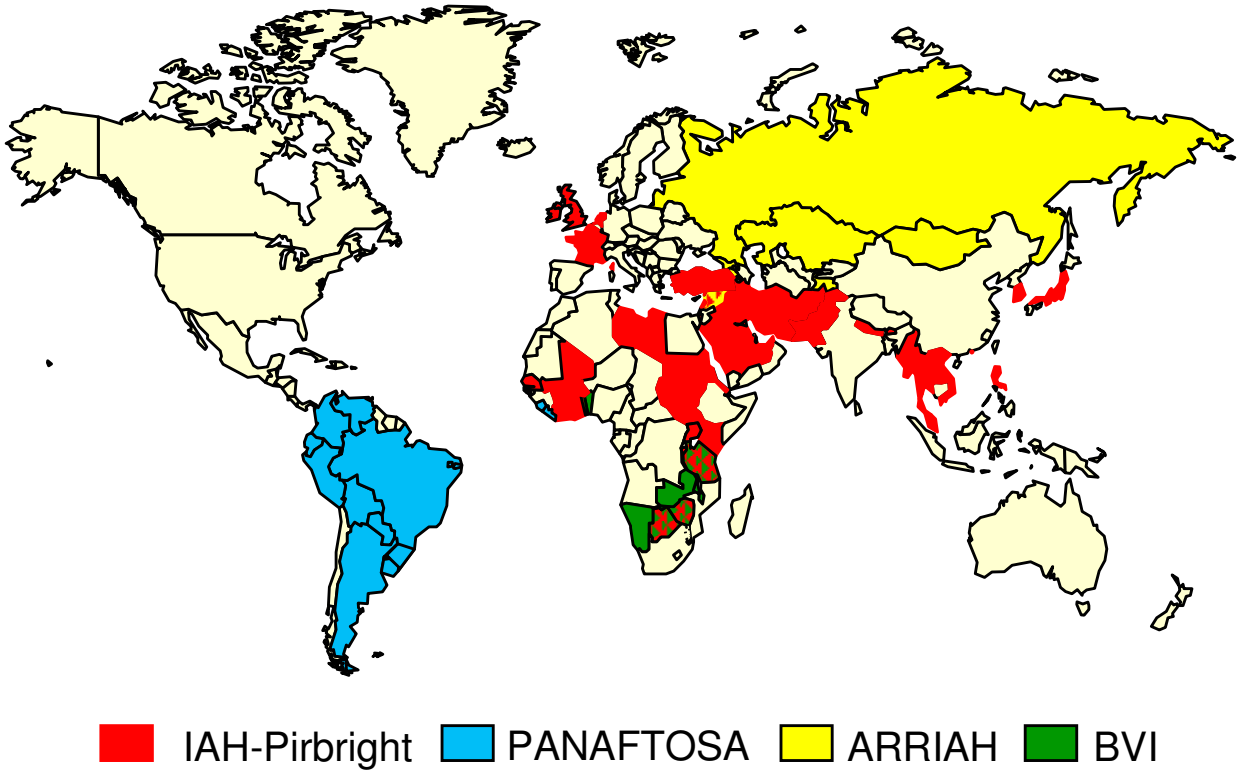
Samples Sent to The FMD WRL in 2005

Country	No. of	Virus isolation in cell culture/ELISA									RT-PCR for FMD (or SVD)		
	samples				FMD virus serotypes				SVD viru	NVD	virus (where appropriate)		
		O	A	C	SAT 1	SAT 2	SAT 3	Asia 1			Positive	Negative	Not teste
BOTSWANA	8	-	-	-	-	8	-	-	-	-	8		-
BURKINA FASO	10	-	-	-	-	-	-	-	-	10	-	-	10
COTE D'IVOIRE	6	-	-	-	-	-	-	-	-	6	-	-	6
ERITREA	31	5	-	-	-	-	-	-	-	26	5	26	-
GHANA	4	-	-	-	-	-	-	-	-	4	-	-	4
KENYA	15	-	2	1	1	7	-	-	-	4	15	-	-
MALI	20	3	1	-	-	-	-	-	-	15	5	15	
TANZANIA	21	6	-	-	-	5	-	-	-	10	12	9	-
TOGO	17	5	1	-	-	-	-	-	-	11	-	3	14
ZAMBIA	16	-	-	-	6	-	-	-	-	10	-	7	9
SUDAN	3	3	-	-	-	-	-	-	-	-	-	2	1
HONG KONG	17	8	-	-	-	-	-	8	-	1	17	-	-
PAKISTAN	36	19	-	-	-	-	-	2b	-	1	35	1	-
MYANMAR	4	4	-	-	-	-	-	-	-	-	4		-
PHILIPPINES	22	15	-	-	-	-	-	-	-	7	11	4	7
THAILAND	9	1	2	-	-	-	-	-	-	6	-	9	-
VIETNAM	11	9	2	-	-	-	-	-	-	-	6	5	-
LAOS	1	-	1	-	-	-	-	-	-	-	-	1	-
IRAN	42	5	13	-	-	-	-	4	-	20	25	5	12
SAUDI ARABIA	14	11	-	-	-	-	-	-	-	3	-	10	4
ITALY	4	-	-	-	-	-	-	-	4	-	4[a]	-	-
IRELAND	4	-	-	-	-	-	-	-	-	4	-	4	-
TOTAL	**311**	**52**	**22**	**1**	**7**	**12**	**-**	**14**	**-**	**134**	**143**	**97**	**67**

Other Intelligence

- Change in reporting to OIE in 2005
- OIE WAHIS system in development
- Visit to Lanzhou Veterinary Research Institute in China and Indian Immunologicals
- Network of FMD

Countries submitting FMDV samples or isolates to different reference laboratories, 2000-2005



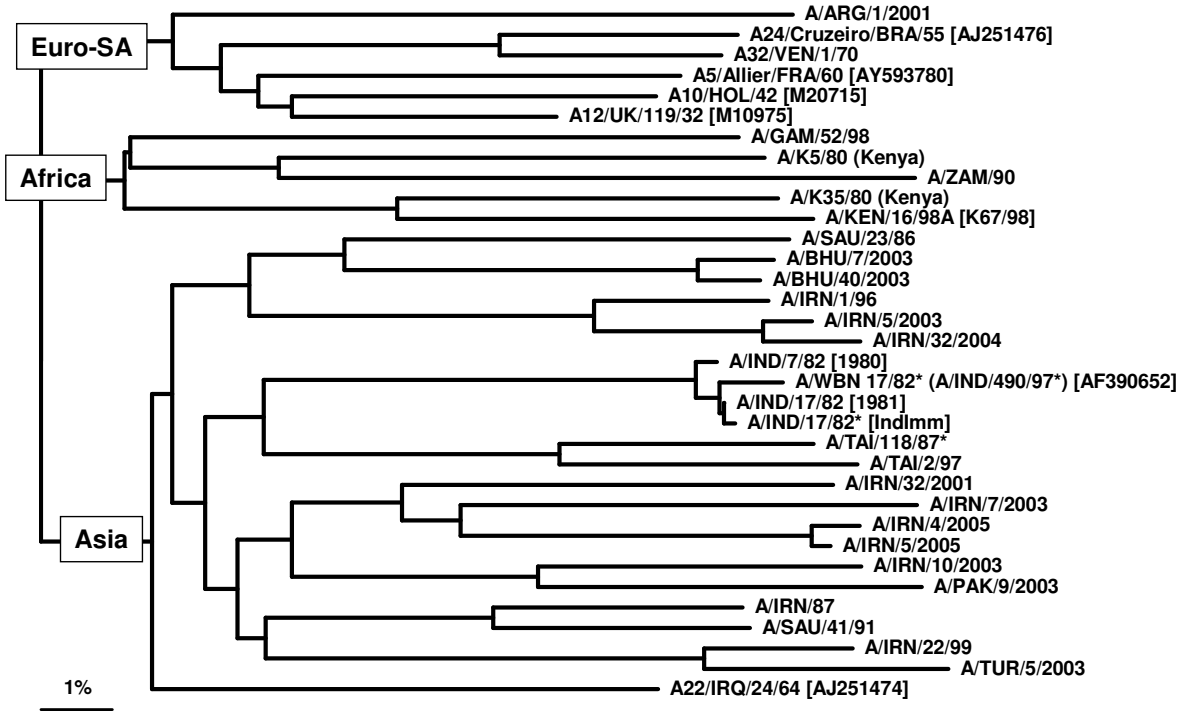
Vaccine matching at different OIE reference laboratories

Reference Laboratory	Mandated by	Average number of samples received annually in last ten years (from how many countries)	Number of field isolates sequenced per annum	Number of vaccine strains routinely matched to field isolates	Vaccine matching tests in routine use
IAH-Pirbright	OIE, FAO	526 (31)	180	~30**	r1 values by liquid phase blocking ELISA and by VNT
PANAFTOSA	OIE, FAO, RIMSA*	46 (9)	30	3	r1 values by by CFT and VNT, and EPP
ARRIAH	OIE	50 (5)	6	9	r1 values by liquid phase blocking ELISA and by VNT
RRL SSA	OIE	50 (6)	35	7	r1 values by VNT

Vaccine requirements by serotype

- Serotype O
  - Much the most important for vaccine antigen reserves
  - Genetically diverse but antigenically fairly restricted. Two main vaccine types
- Serotype A and SATs
  - Genetically and antigenically diverse. Need for multiple vaccine strains
- Serotype Asia 1
  - Antigenically homogeneous. One vaccine
- Serotype C
  - Should we be vaccinating at all?
- SAT 1 still prevalent in parts of S Africa, but not SAT 3

Comparison of the complete VP1 genes of selected FMD A viruses



Neighbor-joining tree of complete VP1 sequences. The three FMDV A topotypes are indicated. The partial VP1 sequence of A/PAK/11/2003 was >99% identical to A/PAK/9/2003.
* Not a WRLFMD reference number.

N.J. Knowles and J.-F. Valarcher, 14 September 2005

Vaccine Matching by Neutralisation

Strain	A22 Iraq	A24 Cruz	A Irn96	A May97	A Irn87	A Sau95	A Irn2001
A Tur 05/03	0.13					0.13	
A Irn 32/01	0.18	0.05		0.06		0.2	
A Irn 06/02	>1.00	0.05	0.85	0.10			
A Irn 01/05		0.08	0.06	0.14	0.16		
A Irn 04/05	>1.00	0.06	0.11	0.13	0.16	0.18	
A Irn 05/05	0.71	0.07	0.10	0.07	0.17	0.18	
A Irn 07/04	0.67	0.05	0.11	0.09	0.12	0.13	
A Irn 32/04	0.16	100%cpe	0.44	100% cpe	0% fix	0.12	
A Irn 33/04			>1.0		0.12	0.12	
A Irn 05/03	0.13	100%cpe	0.14	0.12			
A Irn 07/03	0.13	0.02	0.09	0.06		0.06	
A Irn 10/03	0.18	0.07	0.39	0.14		0.04	
A Irn 41/03	0.33	0.05	0.35	0.06			
A Pak 77/03		0.11	0	0.13	0.22		
A Pak 09/03	0.1					0.31	
A Pak 11/03	0.1					0.36	
A Bhu 07/03	0.22					0.47	
A Bhu 40/03	0.15					0.40	
A Lao 36/03	0.13	0.06	0.20	0.36	0.25		
A May 03/04			0.35	0.44	0.22	0.19	
A May 04/04			0.20	0.37	0.17	0.09	
A Tai 06/04		0.07	0.17	0.28	0.25		
A Tai 09/04		0.05	0.16	0.26	0.29		
A Vit 04/04		0.10	0.32	0.33	0.11	0.07	
A Vit 05/04		0.07	0.21	0.28	0.23	0.11	
A Ken 01/03	0.26	0.09	0.09	0.05	0.15		
A Ken 02/03	0.28	0.09	0.09	0.07	0.14		

RECOMMENDATIONS FROM THE WRL ON FMD VIRUS STRAINS TO BE INCLUDED IN FMDV ANTIGEN BANKS

High Priority

O Manisa (*covers panasian topotype)*
O BFS or Campos
A24 Cruzeiro
Asia 1 Shamir
A Iran '96
SAT 2 Saudi Arabia *(or equivalent)*
(not in order of importance)

Medium Priority

SAT 2 Zimbabwe
A22 Iraq
AIran 87 or A Saudi Arabia 23/86 ***(or equivalent)***
SAT 1 South Africa
A Malaysia 97 *(or Thai equivalent such as A/NPT/TAI/86)*
A Argentina 2001
O Taiwan 97 *(pig-adapted strain or Philippine equivalent)*
A Iran '99 (not in order of importance)

Low Priority

A15 Bangkok related strain
A87 Argentina related strain
A Eritrea 98
C Noville
SAT 2 Kenya
SAT 1 Kenya
SAT 3 Zimbabwe
A Kenya (not in order of importance)

FOOT & MOUTH DISEASE

(CURRENT SITUATION IN PAKISTAN AND THE REGION)

Dr. Manzoor Hussain
Regional Epidemiologist
GTFS/INT/907/ITA

Regional Workshop on Progressive Control Strategies for Foot and Mouth Disease

Bhur Ban Murree Pakistan
(September 11-13, 2004)

RECOMMENDATIONS

- ***A. Regional Approaches***
- Diseases like FMD can be controlled only if there is a strong regional cooperation and an integrated regional approach keeping in view the nature of the disease and the movement of animals in different countries.

- We strongly support SAARC-GF-TADs Regional initiative that consists of components like regional support unit, regional reference labs, epidemiological network and centre and technical backstopping by OIE and FAO. We recommend that OIE be actively involved in this process and assist in ensuring a strong focus on FMD similar to that undertaken for the SEAFMD model.

- We strongly recommend that GF-TAD also take an initiative similar to the one in SAARC in the central Asian Countries and should include Pakistan, Afghanistan and Iran in this initiative. Italian-FAO project, EU-Pakistan Project and EU-Afghanistan project should develop an overlapping coordination mechanism in this regard. These projects can immediately start sharing the information on epidemiology and control of the TADs in the participating countries.

- ***B. National Approaches***
- Both public and private sectors should be involved in design and implementation of strategies.
- Valid national FMD control strategy will require comprehensive epidemiological information of the disease in each country.

- Effective disease reporting, early warning and rapid response mechanism become progressively more important for effective FMD disease control strategy.

- Availability of relevant and effective vaccine is an important tool for the control of FMD in the region. Cold chain system for vaccine needs to be ensured and country should have a national quality control mechanism other than the manufacturer of the vaccine.

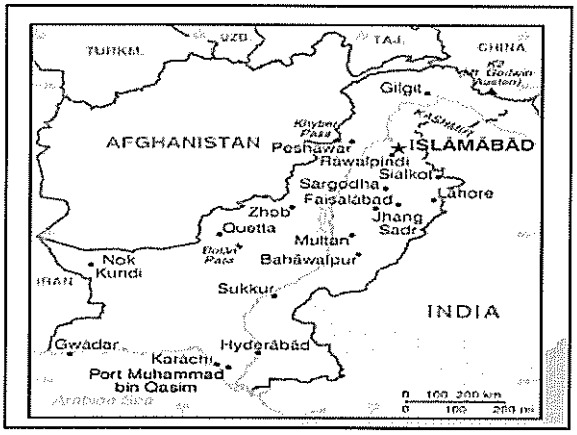
- Diagnostic laboratories and research institutions be appropriately strengthened.
- Awareness and capacity building of different stake-holders for implementation of FMD control should be focused in the strategy.

- There should be a written national policy document for each country which should include emergency preparedness program in case of outbreak. This policy document should be notified.

- The control strategy should emphasize areas which can show early visible benefits to make it more saleable to the policy makers. For Pakistan, the control strategy should first address commercial farming (urban and peri-urban dairying), followed by market-oriented small holders and finally subsistence farmers. Commercial farmers will be willing to share cost for the control strategy and will be more responsive to the efforts being made in this regard.

Regional Project, "Controlling Transboundary Animal Diseases in Central Asian Countries"

- To progress the verification of freedom from Rinderpest
- To better understand the impact of PPR, FMD and other important diseases in the region
- To establish communication between the countries for collaborative disease control
- To establish national disease investigation, control and contingency planning for TADs



FMD in Afghanistan

- The disease present throughout the year
- Surveillance system not efficient
- Minimal diagnostic facilities
- Serotypes A, O and Asia 1 prevalent
- No facility for vaccine production (only imported trivalent vaccine used when available)

FMD in Uzbekistan

- Last laboratory confirmed FMD case (serotype A) in 1991 (year of independence)
- Serotypes A, O reported in past
- Vaccination (6 million) being carried out in bordering areas (Tajikistan, Afghanistan, Kazakhstan)
- Surveillance and diagnostic facilities not satisfactory

FMD in Tajikistan

- Last case reported in 2004 (Asia 1)
- Serotypes A, O, Asia 1 prevalent
- Vaccination from Russia being used in bordering Afghanistan area
- No facilities for confirmation of the disease and typing virus serotypes
- Surveillance system not efficient

FMD in Turkmenistan

- Last case of FMD reported in 2000
- Vaccination in buffer zones (bordering Kazakhstan, Afghanistan, Uzbekistan, Iran)
- Minimal facilities for Lab confirmation
- Veterinary Department being run on self-supporting basis since 1998

FMD in Pakistan

- Every year, outbreaks reported throughout the country
- Serotypes A, O, Asia 1 prevalent
- FMD Referral Lab recently established
- Local vaccine monovalent (O type) and quantity insufficient (0.5-0.8 m doses)
- Dairy colonies, the main source of virus reservoir and transmission
- Severity of the disease increased during last two years

Administrative divisions

- 4 Provinces (Punjab, Sindh, Balochistan, and NWFP),
- One state (AJK), and
- One Capital Territory (Islamabad Capital Territory)
- One Territory (Federally Administered Tribal Areas)

Area

- **Land boundaries:**
  Total: 6,774 km

- *border countries:*
- Afghanistan 2,430 km,
- China 523 km,
- India 2,912 km,
- Iran 909 km

LIVESTOCK IN NATIONAL ECONOMY

- Agriculture in Pak GDP 23.6 %
- Livestock in Pak GDP 11.4 %
- Share in agri GDP 49.1 %
- Livestock in export 8.5 % (935 m US$)
- Provides raw material for industry
- Creates market and capital
- Social security for rural poor
- Security against crop failure in barani areas

- Dependent population > 6.5 m families

Economic Survey (2003-04)

LIVESTOCK POPULATION (2003-04)

(Million Heads)

PROVINCE	CATTLE	BUFFALO	SHEEP	GOAT	CAMEL
PAKISTAN	**23.8**	**25.5**	**24.7**	**54.7**	**0.8**
Per cent distribution					
NWFP	**21.5**	**6.3**	**13.3**	**17.5**	**8.3**
PUNJAB	**43.2**	**60.8**	**24.3**	**37.1**	**18.6**
SINDH	**28.9**	**31.8**	**18.2**	**23.8**	**29.7**
BALOCH-ISTAN	**6.4**	**1.1**	**44.2**	**21.6**	**43.4**

Economic Survey (2003-04)

LIVESTOCK PRODUCTION

- **Milk** **28.624 M ton**
- **Beef** **1.087 M ton**
- **Mutton** **0.723 M ton**
- **Poultry meat** **0.402 M ton**
- **Eggs** **8.247 billion**
- **Wool** **39.7 T ton**
- **Hair** **19.9 T ton**
- **Skins and hides** **48.5 millions**

Economic Survey (2003-04)

Governance of Veterinary Service in Pakistan

- Federal Government
  - Policy, International and Provincial Coordination, Quality Control, R & D in critical areas and Animal Quarantine, Import & Export
- Provincial Governments *(Punjab, Sindh, NWFP, Balochistan, Northern Areas, Azad Jammu & Kashmir)*
  - Provincial policy, Livestock Research & Development, Diagnostic Laboratories and Livestock Extension activities
- District Governments
  - Veterinary Hospitals and Dispensaries

Livestock Disease Status of Pakistan 2004

Disease	Status (# cases)
Rinderpest	Nil
Contagious bovine pleuropneumonia	Never reported
Bovine spongiform encephalopathy	Never reported
Foot & Mouth Disease	+
Peste de Petits Ruminants	4360
Sheep/goat Pox	52001
Haemorrhagic Septicemia	53535
Blackquarter	29404
Contagious caprine pleuropnuemonia	14094
Enterotoxaemia	7895

Rinderpest in Pakistan

- Status
  - Provisional freedom (January 2003)
  - Last case (Sept 2000), No vaccination since Nov 2000
  - Freedom from disease aimed in 2006-7
- Diagnosis
  - Agar gel diffusion
  - ELISA (both antigen & antibody)
  - PCR
  - Virus isolation
- Control
  - Disease search through PDS & Sero-surveillance
  - Vaccine manufacturing facility (1) and Banks (3)
  - Contingency plan in place

Foot & Mouth Disease in Pakistan

- Status
  - Disease is endemic and sporadic in occurrence
  - Severe disease in crossbred & exotic animals
  - Disease is also important in high producing animals
  - Serotypes A, O and Asia 1 with "O" being more prevalent
  - Serotype C reported only once in 1960s
  - The most prevalent disease as per PDS reports
- Diagnosis
  - Complement fixation test
  - ELISA (both antigen & antibody)
  - PCR (under development)
  - Virus isolation (facilities not yet developed)
- Control
  - Notifiable disease
  - Progressive control of disease
  - Vaccine manufacturing facility – limited capacity & quality

Peste de Petits Ruminants in Pakistan

- Status
  - First reported in 1990
  - Is a new emerging disease
  - Sporadic occurrence in selective areas
  - Epidemiology study is under way
- Diagnosis
  - Agar gel diffusion
  - ELISA (both antigen & antibody)
  - PCR (under development)
- Control
  - Current Strategy is disease control
  - Vaccine manufacturing facility (FAO project)
  - Imported vaccine (under FAO project)

- Commercial Dairy Colonies Karachi

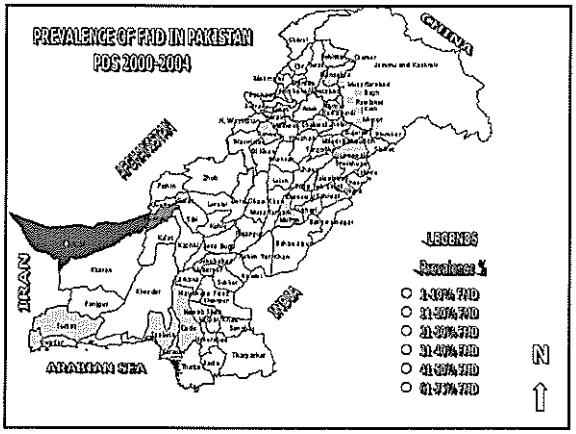
- Landhi Dairy Colonies 200,000
- Bilal Dairy Colony 11,000
- Al-Momin Dairy Society 13,000
- Nagory dairy Society 10,000
- Baldia Town 150,000
- Total Dairy Animals in Karachi: 0.8 million

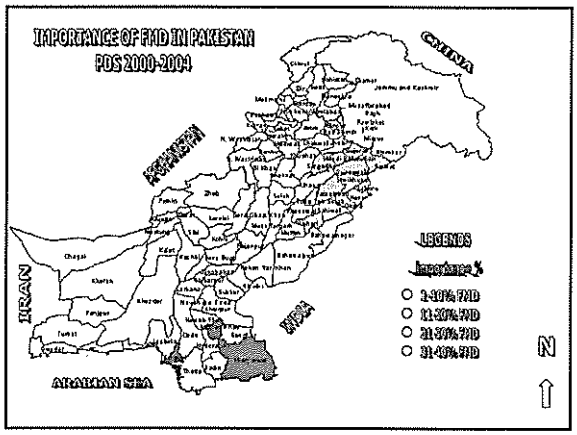
Vaccine Production in the Country

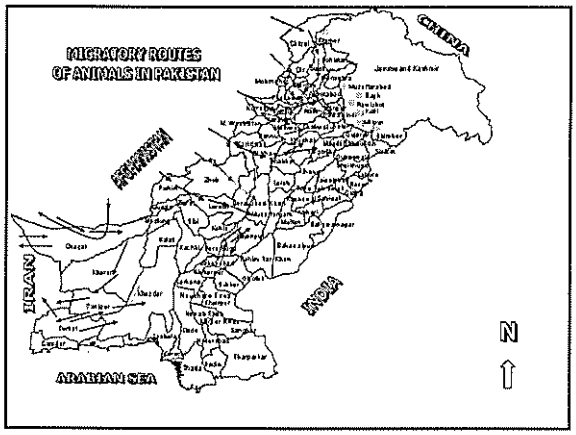
- Vaccines manufactured in both public and private sector (only one for FMD)
- 5 Public sector set-ups
- 5 Private sectors set-ups
- Poultry vaccines also

Priorities in Disease Control

- Control and eradication of diseases of trade and economic importance
  - Freedom from Rinderpest
  - Improved legal framework
  - Control of PPR, FMD
  - Enhanced coverage through vaccination against endemic diseases
- Strengthening of disease diagnostic service
- Capacity building of all stakeholders

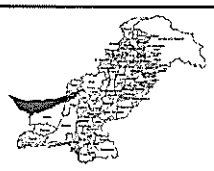




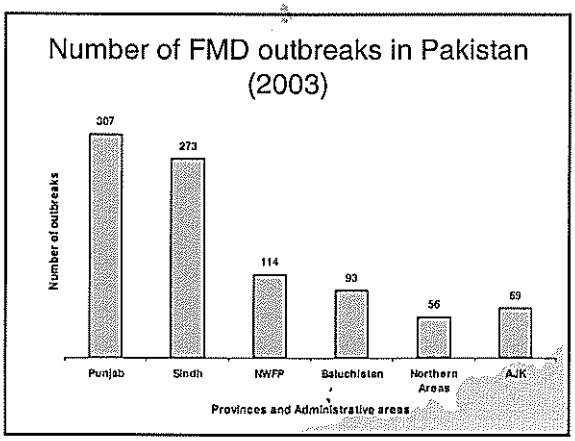


FMD virus sero-types In Pakistan

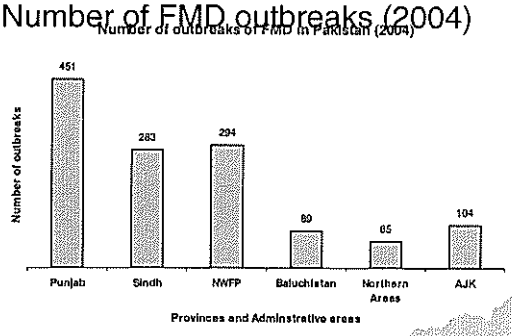
(August 2003 – August 2005)



Province/ Area	Total samples submitted	Total positive	O	A	Asia 1	Improper /not tested
Punjab	109	36*	27	1	9	-
Sindh	100	9	8	1	-	6
NWFP	78	15*	8	2	6	9
Balochistan	20	13	3	9	1	-
ICT	31	5	5	-	-	2
NA	21	14	14	-	1	1
Total	359	92	65	13	17	18



Number of FMD outbreaks (2004)



RECOMMENDATIONS

DIAGNOSTIC FACILITIES

There is a need to establish appropriate facilities for FMD diagnosis and confirmation of virus serotype(s) in the region. ELISA equipment/kits and training is being provided by the Regional Project in all beneficiary countries. It should be supported for further capacity building by the EU Projects in Pakistan and Afghanistan

- SURVEILLANCE

  FMD virus serotype(s) prevalent in the region should be monitored on sustainable basis. The samples should also be sent to the WRL for further analysis.

  The Regional Project will be able to generate and share this information mainly for the beneficiary countries.

- There is considerable movement of animals between Pakistan, Afghanistan and Iran. There is a need that policy makers/scientists from these countries meet on regular basis and based upon the existing knowledge, develop a strategy for the control of FMD in the region

- Epidemiological Analysis

  Very little information is available about the epidemiology of FMD in the region. Efforts are being made by the Regional Project to yield this data in 5 beneficiary countries. However, it is important that this activity is supported and information shared by the countries like Iran, Kyrgyzstan and Kazakhstan to develop a better approach for the control of FMD in the region.

- FMD Vaccine Production

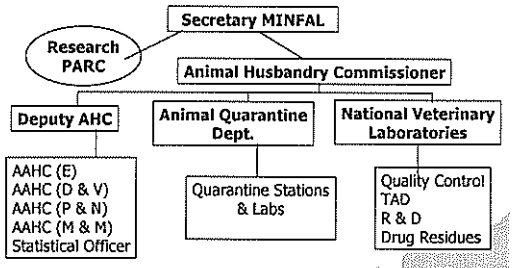
  Quality assured FMD vaccine containing appropriate virus serotype(s) is not produced in the region. Only commercial dairy farmers are buying this vaccine from other multinational companies on a higher price. It is recommended that facilities should be established for the production of quality FMD vaccine at a suitable place in the region.

- Training:

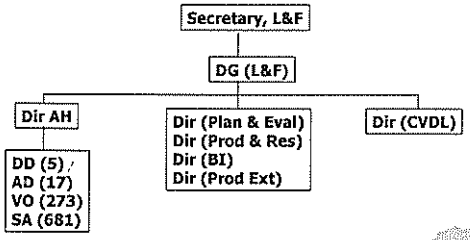
1. Quality testing of vaccines
2. Virus isolation and identification
3. Strengthening of FMD Referral Lab
4. Application of FMD pen-side test??

THANK YOU

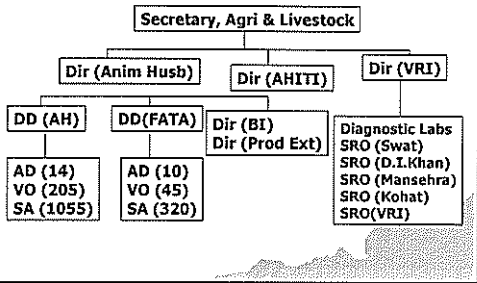
Veterinary Service in Federal Government



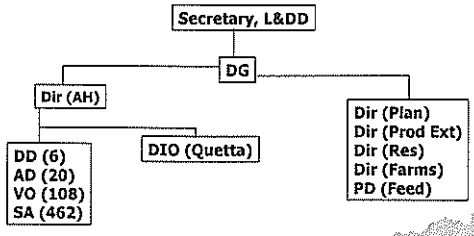
Veterinary Service in Sindh

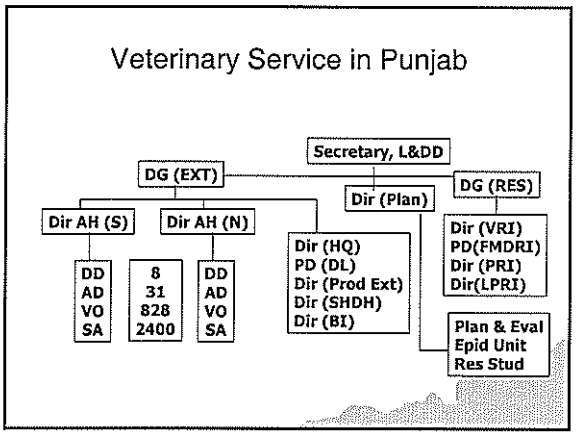


Veterinary Service in NWFP



Veterinary Service in Balochistan



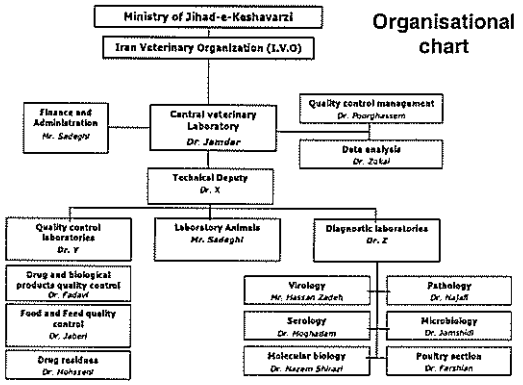

Veterinary Service in Punjab
Secretary, L&DD
DG (EXT)
DG (RES)
Dir (Plan)
Dir AH (S)
Dir AH (N)
Dir (HQ)
PD (DL)
Dir (Prod Ext)
Dir (SHDH)
Dir (BI)
Dir (VRI)
PD(FMDRI)
Dir (PRI)
Dir(LPRI)
DD
AD
VO
SA
8
31
828
2400
DD
AD
VO
SA
Plan & Eval
Epid Unit
Res Stud

Appendix 6

FMD surveillance in Iran - information provided by the Central Veterinary Laboratory, Karaj, Iran

M. JAMDAR 12/09/2005

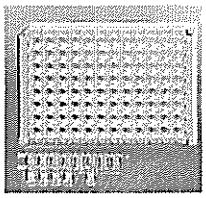
Organisational chart



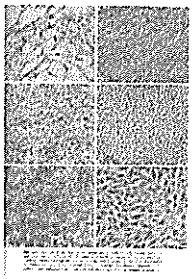
FMDV diagnostic methods used at CVL

- ✓ Immunocapture Elisa (Antigen Elisa)
- ✓ Virus Isolation (cell culture BHK 21)
- ✓ Molecular biology (RT-PCR)

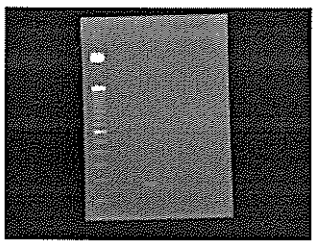
Antigen detection ELISA



Virus isolation (*cell culture BHK 21*)

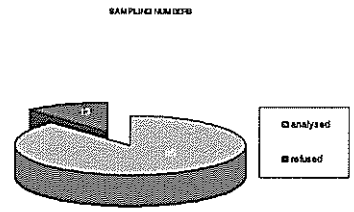


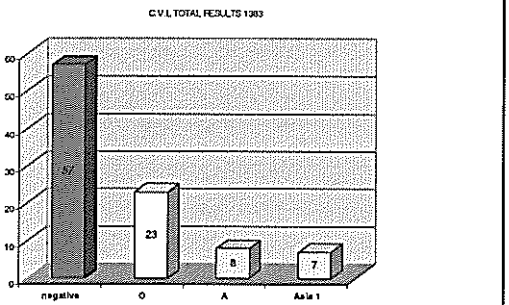
Molecular biology (*RT-PCR*)

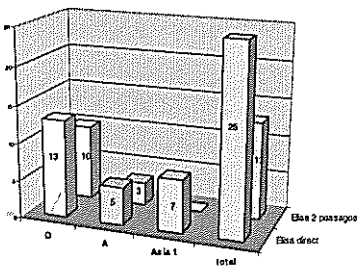


1383 results (from 03/2004 to 03/2005)

12/09/2005

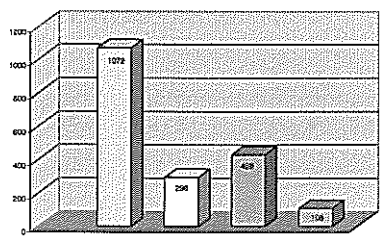


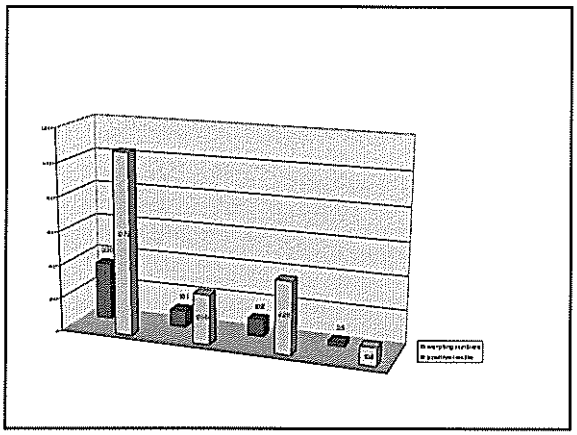


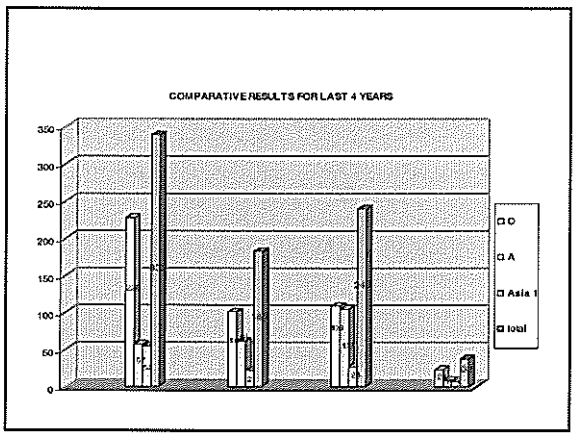


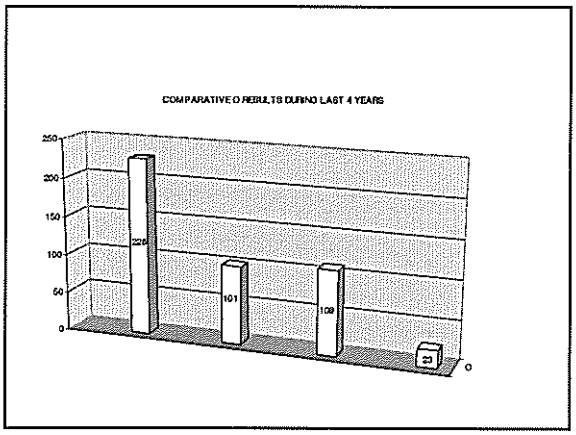
1380 / 1383 analysis
(4 year period, from 03/2001to 03/2005)

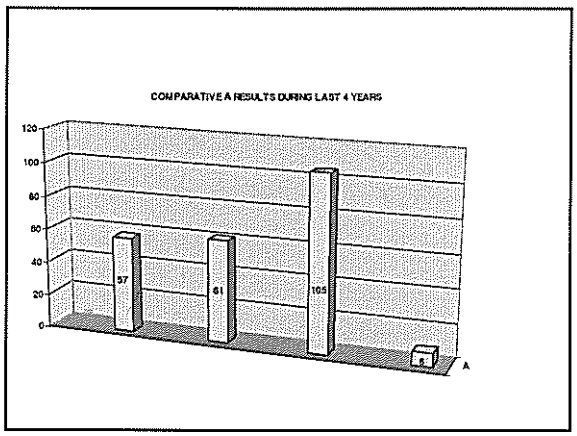
12/09/2005

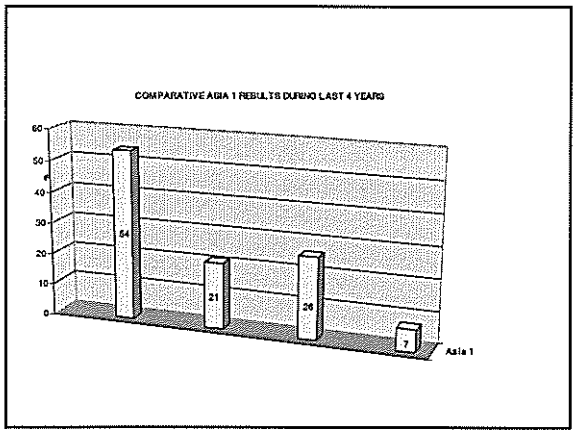








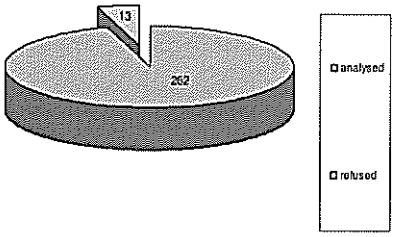




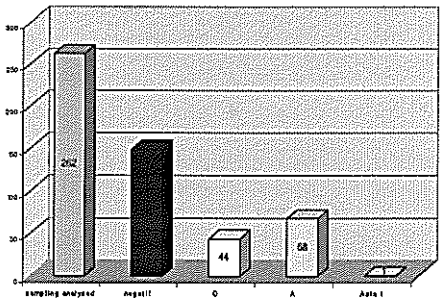
1384 results (from 20/3/2005 to 20/08/2005)

M JAMDAR 12/09/2005

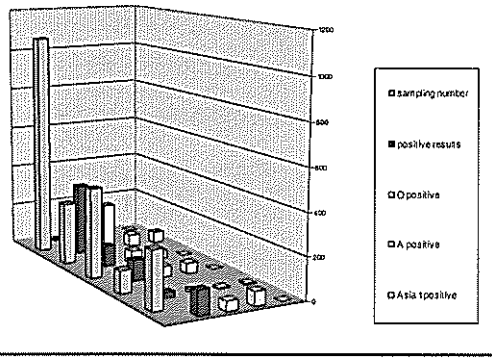
SAMPLING ANALYSED (nb)



GLOBAL RESULTS APRIL - AUGUST 2005

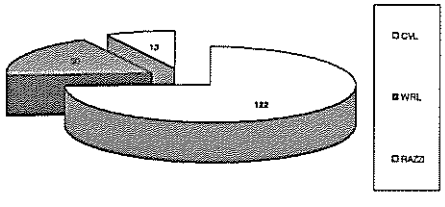


1380 / 81 / 82 / 83 / 84 (*-5 first months of 1384*)

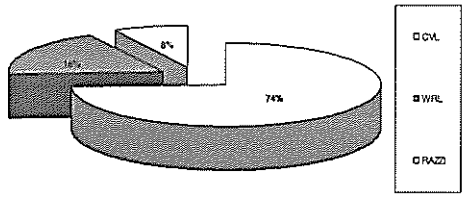


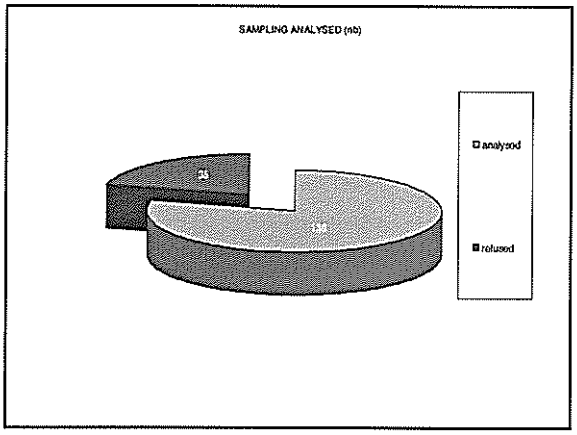
FMD surveillance in Iran - information provided by the Iran Veterinary Organisation

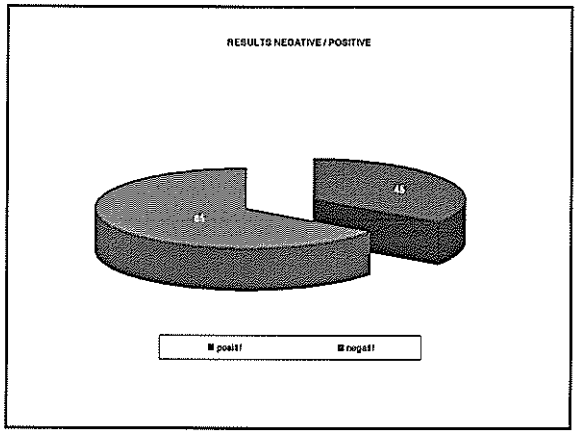
SAMPLING DISTRIBUTION IN CVL, WRL and RAZZI INSTITUTE (nb)

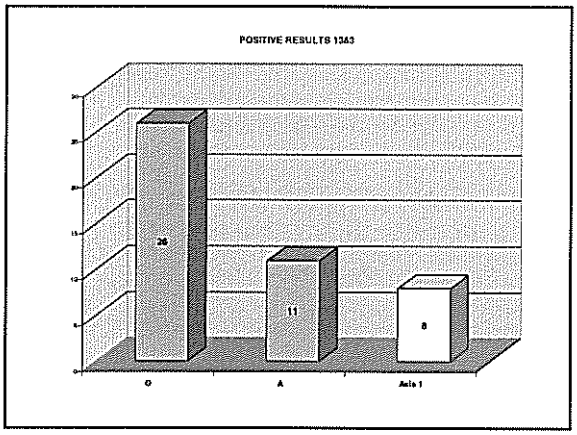


SAMPLING DISTRIBUTION IN CVL, WRL and RAZZI INSTITUTE (%)

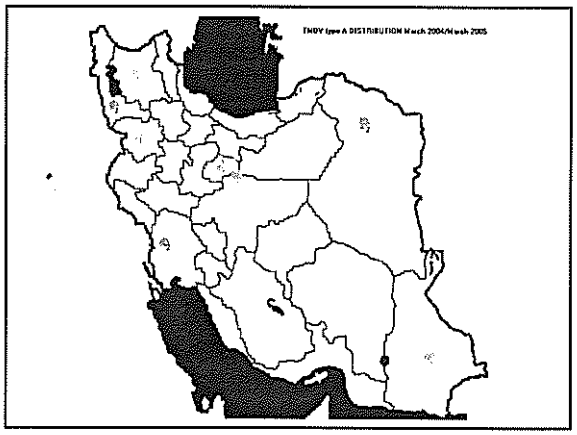


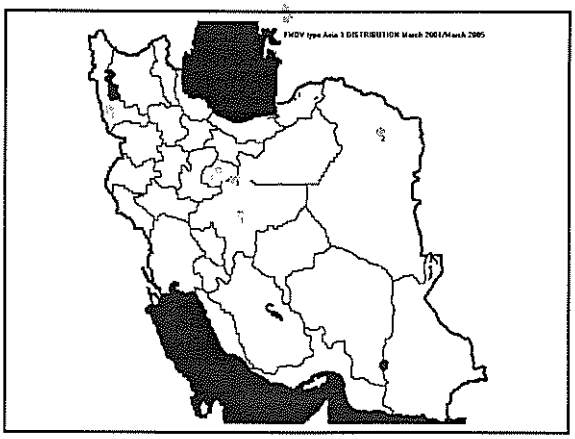

SAMPLING ANALYSED (nb)
analysed
refused


RESULTS NEGATIVE / POSITIVE
45
85
posit
negat


POSITIVE RESULTS 1383
26
11
8
O
A
Asia 1


FMDV type O DISTRIBUTION March 2004/March 2005


FMDV type A DISTRIBUTION March 2004/March 2005


FMDV type Asia 1 DISTRIBUTION March 2004/March 2005

Appendix 7

A Serosurveillance for FMDV-NSP in Juvenile Cattle in Turkish Thrace, 2005

BULUT, A.N., SAREYYUPOGLU, B., TEZEL, A. AND AKTAS, S.

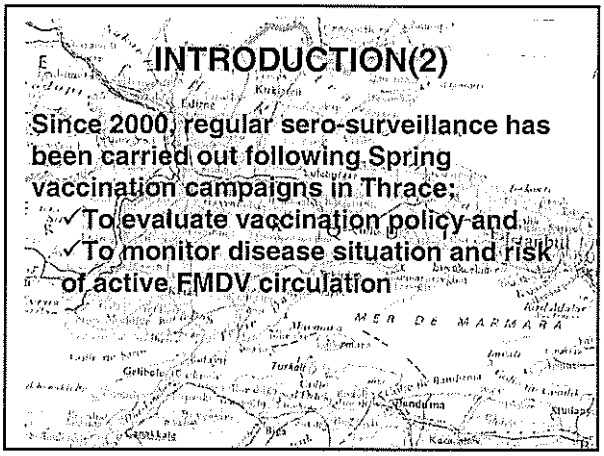
Sap Institute, ANKARA, TURKEY

Introduction(1)

FMD Vaccination Programme in Thrace

- ✓ CATTLE: Spring & Autumn vaccination
- ✓ SRs: Spring only
- ✓ Şap Institute Trivalent vaccine (O/A/Asia1)

INTRODUCTION(2)



Since 2000, regular sero-surveillance has been carried out following Spring vaccination campaigns in Thrace;

- **✓To evaluate vaccination policy and**
- **✓To monitor disease situation and risk of active FMDV circulation**

INTRODUCTION(3)

A sero-surveillance was carried out again this year but the aim and design of the serosurvey was different from previous serosurveys:

The primary objective was to provide evidence that FMD virus has not been circulating

- ✓ focus exclusively on cattle
- ✓ target juvenile cattle *(< 2 years but >4 months)*
- ✓ two-stage sampling strategy: first sampling *at the time of vaccination*
- ✓ *and follow-up investigation*
- ✓ villages as primary sampling units
- ✓ detect a 2% prevalence of "infected villages"
- ✓ detect a 5% within-village prevalence
- ✓ require 95% confidence in result

The secondary objective was evaluation of the field efficacy of Şap Institute vaccine

INTRODUCTION(4)

Primary objective to provide evidence that FMD virus has not been circulating

- ✓ Select 152 villages & 64 cattle per village = 9728 cattle
- ✓ Test all sera by NSP Ab ELISA

Secondary objective to demonstrate field efficacy of Şap Institute vaccine

- ✓ 15/152 villages resampled at 60 dpv = 960 cattle
- ✓ Paired sera of each tested by LPBE (x3 serotypes)
- ✓ *If seropositive at 1:100, is the animal "protected"*

Material and Method

1. Sera
   - ✓ NSP-ELISA sera: In total 9728 sera were collected at 0. day during the vaccination
   - ✓ Sera for measuring antibody level: 960 sera were collected at 60. day postvaccination in 15/152 units.
   - ✓ Follow-up investigation sera: In total 477 sera were collected in 8 positive units.
     - ☞Villages, Kayabaşı, Çiftalan and Frizköy in Istanbul, were sampled as positive premises (all cattle in each premise) and within village population as randomly with 2% prevalance and 95% confidence.
     - ☞In the others units, just all animals were bled in positive premises.
2. Oeso-pharyngeal fluid (OP)
   - ✓ During the follow-up investigation, 34 OP were collected from NSP positive cattle.

Material and Method (2)

3. ELISAs
   - ✓ NSP ELISA:

   CEDI-diagnostic NSP-FMD ELISA kit was used for primary testing of sera and sera which were detected as positive by CEDI were tested again by Bomelli-chekit NSP ELISA kit.
   - ✓ Antibody detection ELISA:

   Liquid-phase Blocking ELISA (LPBE) was used for measuring of antibody levels and titration of sera detected as positive by NSP ELISAs.
   - ✓ Antigen detection ELISA:

   Indirect Sandwich Antigen Detection ELISA was used for testing of tissue culture supernatants for OP fluids.
4. Primary cell cultures
   - ✓ Lamb Kindney Primary cell cultures were used for virus isolation from OPs.

RESULTS

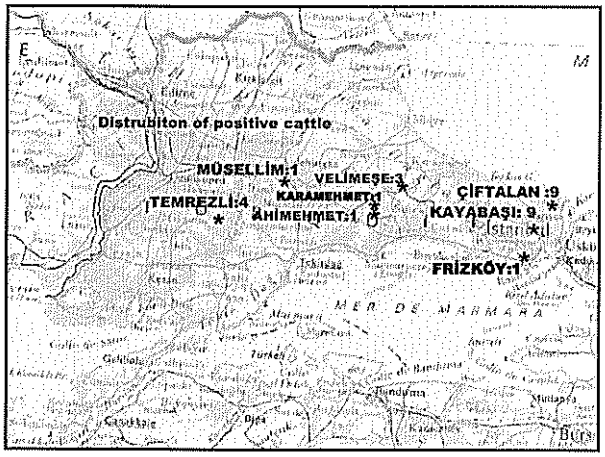
1.Results of the main survey

- ✓ 29 sera were positive in total 9728 sera by NSP-FMD ELISA(CEDI-Diagnostic)
- ✓ Positive sera were tested again by NSP-ELISA Bomelli-chekit
- ✓ And titrated by LPBE

PROVINCE	NUM.OF SERA	NUM. OF POSITIVE	%
ÇANAKKALE	320	0	0
EDİRNE	2624	0	0
İSTANBUL	1600	19	1.187
KIRKLARELİ	2304	1	0.04
TEKİRDAĞ	2880	9	0.31
TOTAL	9728	29	0.29

Results of the NSP-FMD Survey: Distribution of Positive Sera by Villages

PROVINCE	DISTRICT	VILLAGE	NUM. OF POSITIVE
İSTANBUL	B.ÇEKMECE	FİRİZKÖY	1
İSTANBUL	B.ÇEKMECE	KAYABAŞI	9
İSTANBUL	EYÜP	ÇİFTALAN	9
KIRKLARELİ	BABAESKİ	MÜSELLİM	1
TEKİRDAĞ	ÇORLU	KARAMEHMET	1
TEKİRDAĞ	ÇORLU	AHİMEHMET	1
TEKİRDAĞ	ÇORLU	VELİMEŞE	3
TEKİRDAĞ	HAYRABOLU	TEMREZLİ	4
		TOTAL	29



2. Antibody level

In total, 70,6%, 73,6% and 69,3%protection rates were detected for types O, A and Asia-1 respectively from sera collected at day 60 postvaccination.

		PROTECTION RATE (%)					
		TYPE O		TYPE A		TYPE ASIA-1	
PROVINCE	NUM.of SERA	04-12(*)	12-24	04-12	12-24	04-12	12-24
ÇANAKKALE	192	62	81	65	82	58	79
EDİRNE	192	64	79	64	85	61	78
İSTANBUL	192	52	75	55	77	54	74
KIRKLARELİ	192	63	78	69	83	59	80
TEKİRDAĞ	192	70	82	71	85	68	82
TOTAL	960	62,2	79	64,8	82,4	60	78,6
	(*)= age of the cattle(Month)						

Follow-up investigation results

VILLAGES	POPULATION SIZE	NUM.of SERA	NUM. of POSITIVE	NUM. Of PREVIOUS POSITIVITY	N.B.
FRIZKÖY	172	65	4	1	
KAYABAŞI	960	149	26	9	
ÇİFTALAN	320	58	5	9	same animals positive
AHİMEHMET	585	32	1	1	same animals positive
KARAMEHMET	1232	36	1	1	same animals positive
VELİMEŞE	800	45	1	3	
TEMREZLİ	400	47	4	4	same animals positive
MÜSELLİM	252	45	0	1	
TOTAL	4721	477	42	29	

Follow-up investigation results(2)

Kayabaşı:

- Population size:960
- Number of sera tested:149
- Number of NSP positivity: 26
- An unreported outbreak was detected.
- Outbreak was occurred after the Kurban Festival within two premises (End of January/beginning of February).
- All 26 positive sera were from these two premises.
- And no other positive animals found in other premises within this village.

Follow-up investigation results(3)

The other villages (Frizköy, Çiftalan, Velimeşe, Ahimehmet, Karamehmet, Temrezli and Müsellim):

- Total positivity:16
- Although sampling of juvenile cattle was required, it was identified that the positive sera were taken from older cattle in the main survey.
- Because of this, some animals were scored as positive.
- During the follow-up investigation in addition to these animals all animals within these premises were sampled.
- Except from those previously positive animals, there was no positive animals from these sampling (from young and older cattle)
- So it is concluded that there is no active FMDV circulation in these villages.

Discussion

- This year more animals were bled (in Thrace and also in each unit: last year 4800/48; this year 9728/64).
- Only young cattle were sampled.
- Last year it was carried out after vaccination, but this year sera were collected at day 0.
- Although the amount of sera have been doubled, the positivity rate was low when compared to those of previous years (1.18%/0.29%)

Appendix 8

EPIDEMIOLOGICAL INVESTIGATION OF FMD IN ERZURUM

PROGRESS REPORT

Dr. Sinan AKTAS

WHY ERZURUM ?

- 25.066 km²
  - 64% pasture
- Population: 950.000
- Cattle population: 550.000
  - 4% European breeds
  - 36% Cross breeds
  - 60% Local breeds

WHY ERZURUM ?

- Highest FMD occurence
- Very low vaccination coverage
- Biggest animal market in Turkey
- Animal distribution center for Turkey
- Located on an important transport route between Eastern and Western Turkey

PROGRESS

- 79 of 98 villages visited so far.
- About 500 people were interviewed in these 79 villages.
- A total of 6468 cattle owners present in these villages.
- Total cattle population of these villages was about 83.920.
- 4 active FMD outbreaks determined.

PROGRESS

- More than 20 villages were told to have had FMD in 2005.
- Sera samples collected or will be collected from these villages for laboratory studies.
- 14 villages had FMD in 2004.
- 15 villages had FMD in 2003.

PROGRESS

- Animal market in Erzurum seen as the major source of FMD.
- Most of the villages, they are not against vaccination.
- 90% of the disease has been observed in unvaccinated and young animals.
- They are reluctant to report the disease.

Quality Assurance of FMD Virus Detection and Serology

David Paton, Donald King, Satya Parida, Nigel Ferris

Aspects of Quality Assurance

- Use of properly validated test
- Reference standards
  - To calibrate tests
- Proficiency Panels
  - To evaluate performance (EQA)
- Internal quality procedures
  - To monitor routine performance

Talk Overview

- Pilot proficiency test for VI and RT-PCR
- Progress on serological test validation and preparation of QA materials

Ring test for FMDV detection methods

- Initial pilot

Institute for Animal Health – UK
CODA-CERVA-VAR -Belgium
Friedrich-Loeffler Instutute - Germany
Istituto Zooprofilattico Sperimentale Della Lombardia - Italy
Central Institute for Animal Disease Control -The Netherlands

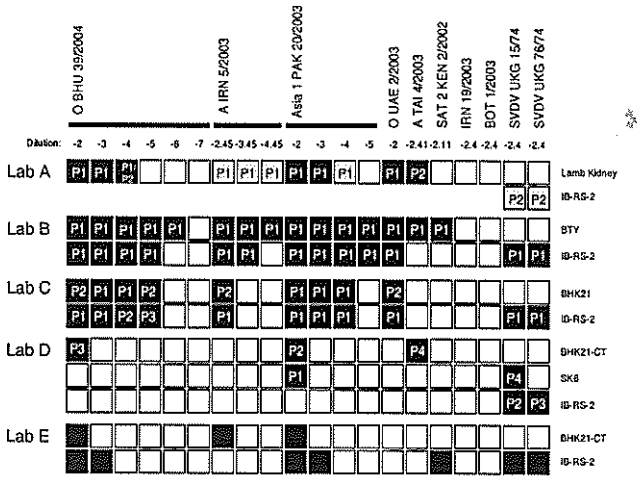
- Aims of pilot

1. To test "logistics" of generating and dispatching samples
2. Identify material suitable for long-term inter-laboratory comparative exercises
3. Generate comparative data for the performance of VI and RT-PCR in different laboratories

- 20 coded samples

-Epithelium samples (comprising O, A, Asia 1, SAT2 and SVDV)
-3 10-fold titration series
-2 NVD samples (however, no samples derived from known negative animals)

Virus isolation results:



Virus isolation methods:

Laboratory number	A		B		C		D			E	
Cell culture	Lamb kidney	IB-RS-2	Bovine thyroid (BTY)	IB-RS-2	IB-RS-2	BHK21	BHK21-CT	SK6	IB-RS-2	BHK21-CT	IB-RS-2
Culture flasks	6-well plates	6-well plates	Tubes	Tubes	12-well plates	12-well plates	24-well plates	24-well plates	24-well plates	Plaque dishes	12.5 cm^2 flasks
Culture medium	Earle's	Earle's	Eagle's	Eagle's	MEM 2% FCS	MEM 2% FCS	MEM	LM medium	MEM	MEM	MEM
Volume of sample added (ml)	0.2	0.2	0.2	0.2	0.5	0.5	0.3	0.3	0.3	0.1	0.1
Number of cell culture replicates	3	3	5	5	1	1	2	2	2	1	1
Sample adsorption	37 °C 60 min	37 °C 60 min	37 °C 30 min	37 °C 30 min			37 °C 30 min	37 °C 30 min	37 °C 30 min		
Culture	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h
Number of blind passages	1	1	1	1	2	2	3	3	3	1	1
CPE confirmation by antigen ELISA using	Polyclonal antisera		Polyclonal antisera		Monoclonal antibodies		Polyclonal antisera			Polyclonal antisera	

RT-PCR methods:

	Laboratory number	1			2			3		4	5
	FMDV test number	1	2	3	4	5	6	7	8	9	10 - 11
	Serotype	All 7	All 7	All 7	All 7	All 7	Type O	All 7	All 7	All 7	All 7
	Assay	Real-time	Real-time	Real-time	Real-time	Conventional	Conventional	Conventional	Real-time	Real-time	Conventional
	Targeted genomic region	5'-UTR	3D	5'-UTR	5'-UTR	2B	1D	3D?	3D	3D	3D
RNA extraction	RNA extraction method	Total NA kit (Roche)	Total NA kit (Roche)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	Phenol/ Chloroform/ Isopropanol	Phenol/ Chloroform/ Isopropanol	Total NA Kit (Roche)	RNAeasy kit (Qiagen)
	RNA extraction equipment	MagNA Pure LC (Roche)	MagNA Pure LC (Roche)	manual	manual	manual	manual	manual	manual	MagNA Pure LC (Roche)	manual
	Sample lysis buffer	Trizol (Invitrogen)	Trizol (Invitrogen)	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Trizol (Invitrogen)	Trizol (Invitrogen)	None	Buffer RLT supplied with kit
	Sample volume (µl)	33	33	140	140	140	140	250	250	200	100
	Elution volume (µl)	100	100	60	60	60	60	50	50	100	30
Reverse Transcription	RT buffer	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Titan One-tube RT-PCR (Roche)	Titan One-tube RT-PCR (Roche)	Superscript III (Invitrogen)	Superscript III (Invitrogen)	LightCycler (Roche)[d]	SOURCE Roche?
	Volume of RNA in RT mix (µl)	6	6	9.625	7.5	2	2	10	5	9	6
	Total RT mix volume (µl)	15	15	25	25	10	10	40	25	20	20
	RT enzyme	MMLV	MMLV	MMLV	MMLV	AMV	AMV	MMLV	MMLV	Tth DNA pol	AMV
	RT Incubation (°C)	48° (45 mins)	48° (45 mins)	25° (10 min) 48° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	61° (20 mins)	42° (60 mins)
	Inactivation step	95° (5 mins)	95° (5 mins)	95° (5 mins)	See PCR below	94° (2 mins)	94° (2 mins)	94° (2 mins)	94° (2 mins)	95° (30s)	96° (3 mins)
Polymerase chain reaction	PCR buffer	Universal TaqMan (ABI)	Universal TaqMan (ABI)	Universal TaqMan (ABI)	Universal TaqMan (ABI)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	in-house buffer
	Volume of cDNA added (µl)	7	7	10	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one step)	5
	Total PCR vol (µl)	25	25	25	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	20
	PCR primers (pmol)	22.5	22.5	22.5	22.5	10	10	16	20	6	10
	Probe (pmol)	7.5	7.5	5	7.5	-	-	-	4	3	-
	Cycling parameters	50° (120s) | 95° (600s) | 95° (15s) 60° (60s) ↓↑ *50 cycles*	50° (120s) | 95° (600s) | 95° (15s) 60° (60s) ↓↑ *50 cycles*	95° (600s) | 95° (15s) 60° (60s) ↓↑ *45 cycles*	95° (600s) | 95° (15s) 60° (60s) ↓↑ *45 cycles*	95° (15s) 52° (30s) 72° (45s) ↓↑ *30 cycles* | 72° (7 mins)	95° (15s) 52° (30s) 72° (45s) ↓↑ *30 cycles* | 72° (7 mins)	94° (30s) | 58° (30s) 68° (60s) ↓↑ *40 cycles* | 72° (10 mins)	94° (30s) 55° (30s) 68° (60s) ↓↑ 42 *cycles*	95° (1s) 60° (15s) 72° (13s) ↓↑ *45 cycles* | 40° (15s)	96° (300s) | 96° (40s) 62° (40s) 72° (50s) ↓↑ *40 cycles* | 72° (7 mins)
	Thermocycler	ABI 5700	ABI 5700	ABI 7900HT	ABI 7900HT	Perkin Elmer GeneAmp 2400	Perkin Elmer GeneAmp 2400	Biometra	Stratagene Mx3000p	LightCycler I.2	Perkin Elmer GeneAmp 9700

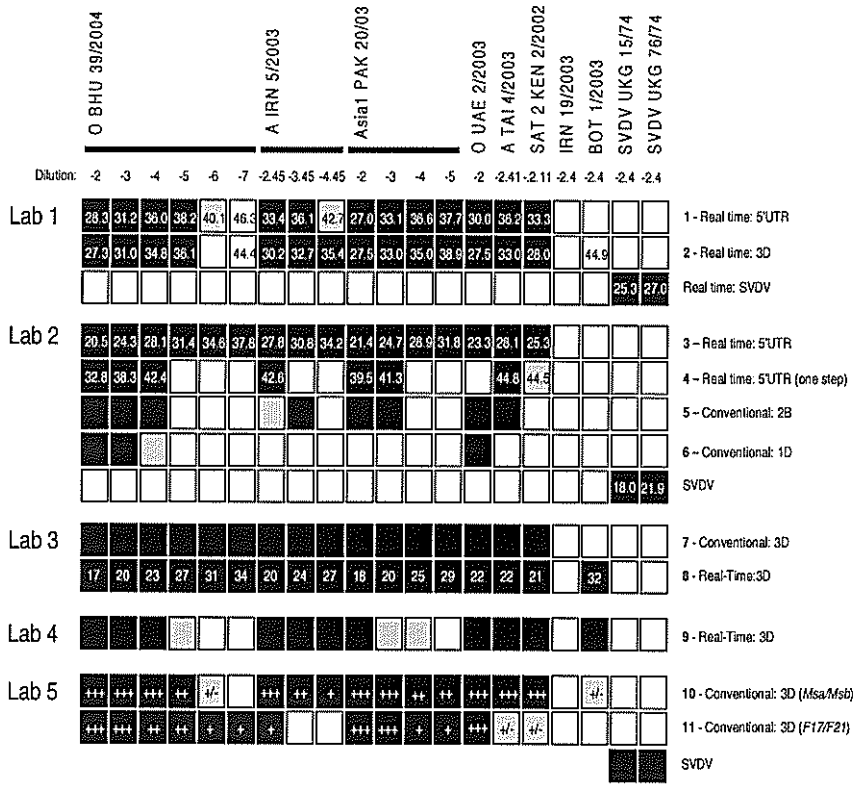
Single-tube assay formats

RT-PCR methods:

	Laboratory number	1			2			3		4	5
	FMDV test number	1	2	3	4	5	6	7	8	9	10 - 11
	Serotype	All 7	All 7	All 7	All 7	All 7	Type O	All 7	All 7	All 7	All 7
	Assay	Real-time	Real-time	Real-time	Real-time	Conventional	Conventional	Conventional	Real-time	Real-time	Conventional
	Targeted genomic region	5'-UTR	3D	5'-UTR	5'-UTR	2B	1D	3D?	3D	3D	3D
RNA extraction	RNA extraction method	Total NA kit (Roche)	Total NA kit (Roche)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	QIAamp® Viral RNA Mini Kit (Qiagen)	Phenol/ Chloroform/ Isopropanol	Phenol/ Chloroform/ Isopropanol	Total NA Kit (Roche)	RNAeasy kit (Qiagen)
	RNA extraction equipment	MagNA Pure LC (Roche)	MagNA Pure LC (Roche)	manual	manual	manual	manual	manual	manual	MagNA Pure LC (Roche)	manual
	Sample lysis buffer	Trizol (Invitrogen)	Trizol (Invitrogen)	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Buffer AVL supplied with Kit	Trizol (Invitrogen)	Trizol (Invitrogen)	None	Buffer RLT supplied with kit
	Sample volume (µl)	33	33	140	140	140	140	250	250	200	100
	Elution volume (µl)	100	100	60	60	60	60	50	50	100	30
Reverse Transcription	RT buffer	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Multiscribe (ABI)[a]	Titan One-tube RT-PCR (Roche)	Titan One-tube RT-PCR (Roche)	Superscript III (Invitrogen)	Superscript III (Invitrogen)	LightCycler (Roche)[d]	SOURCE Roche?
	Volume of RNA in RT mix (µl)	6	6	9.625	7.5	2	2	10	5	9	6
	Total RT mix volume (µl)	15	15	25	25	10	10	40	25	20	20
	RT enzyme	MMLV	MMLV	MMLV	MMLV	AMV	AMV	MMLV	MMLV	Tth DNA pol	AMV
	RT Incubation (°C)	48° (45 mins)	48° (45 mins)	25° (10 min) 48° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	50° (30 mins)	61° (20 mins)	42° (60 mins)
	Inactivation step	95° (5 mins)	95° (5 mins)	95° (5 mins)	See PCR below	94° (2 mins)	94° (2 mins)	94° (2 mins)	94° (2 mins)	95° (30s)	96° (3 mins)
Polymerase chain reaction	PCR buffer	Universal TaqMan (ABI)	Universal TaqMan (ABI)	Universal TaqMan (ABI)	Universal TaqMan (ABI)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	in-house buffer
	Volume of cDNA added (µl)	7	7	10	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one step)	5
	Total PCR vol (µl)	25	25	25	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	(n.a., one-step)	20
	PCR primers (pmol)	22.5	22.5	22.5	22.5	10	10	16	20	6	10
	Probe (pmol)	7.5	7.5	5	7.5	-	-	-	4	3	-
	Cycling parameters	50° (120s) | 95° (600s) | 95° (15s) 60° (60s) ↓↑ *50 cycles*	50° (120s) | 95° (600s) | 95° (15s) 60° (60s) ↓↑ *50 cycles*	95° (600s) | 95° (15s) 60° (60s) ↓↑ *45 cycles*	95° (600s) | 95° (15s) 60° (60s) ↓↑ *45 cycles*	95° (15s) 52° (30s) 72° (45s) ↓↑ *30 cycles* | 72° (7 mins)	95° (15s) 52° (30s) 72° (45s) ↓↑ *30 cycles* | 72° (7 mins)	94° (30s) | 58° (30s) 68° (60s) ↓↑ *40 cycles* | 72° (10 mins)	94° (30s) 55° (30s) 68° (60s) ↓↑ 42 *cycles*	95° (1s) 60° (15s) 72° (13s) ↓↑ *45 cycles* | 40° (15s)	96° (300s) | 96° (40s) 62° (40s) 72° (50s) ↓↑ *40 cycles* | 72° (7 mins)
	Thermocycler	ABI 5700	ABI 5700	ABI 7900HT	ABI 7900HT	Perkin Elmer GeneAmp 2400	Perkin Elmer GeneAmp 2400	Biometra	Stratagene Mx3000p	LightCycler I.2	Perkin Elmer GeneAmp 9700

Single-tube assay formats

RT-PCR results:



Next phase.....

Goals:

1: External Quality Assurance

blind challenge of routine diagnostic assays

⇩

Equivalence testing?

demonstrate that routine assays used at different labs have similar ability to detect FMDV

2: Generate standards available to reference laboratories

used to calibrate controls run in these routine diagnostic assays

Planned for Winter 2005:

- Expand number of participating laboratories
- Modify panel to include epithelium samples from "known" negatives

Questions?

- Should we send a separate panel for labs without the facilities to handle live FMDV (RT-PCR only panel? - if so what form should the samples be in – TRIZOL, RNA etc..)
- Should antigen-ELISA be included/covered by separate panel?

Serology Proficiency – up to now

- **Phase XVIII – completed in 2004**

New reference sera for A Iran 96, O SKR, Asia 1 Shamir
Proficiency test panel
SPCE pilot study
Reported in Crete meeting 2004

- **Phase XIX – planned for 2005 & 2006**

Annual proficiency panel in each of 2005 & 2006
Distribute positive pig and sheep sera for use in NSPE
Generate SAT 2 sera

- **What has been done in 2005**

Proposal and costing prepared, but no Phase XIX contract signed and no sera distributed
SAT 2 sera prepared in bulk from vaccine potency study
NSP bovine serum panel prepared and paper submitted to OIE Sci et Tech Rev
Discussions with EQA specialist provider over provision of assistance
Paper submitted to Veterinary Record on Zimbabwe field study
Collection and analysis of Hong Kong pig sera

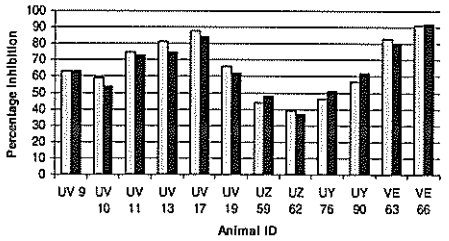
NSP serum panel

38 cattle sera
100-800 ml of each
O, A, Asia 1
1-6 months post challenge
Carriers/non-carriers
Vaccinated/not vaccinated

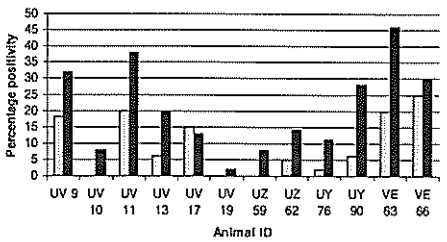
Animal ID	Vaccine strain used	Challenge exposure route (DC, direct contact; I, inoculation)	Challenge exposure virus	Clinical signs	Last day after challenge that infection detected	Carrier status (C, carrier; -, non carrier)	Days after challenge that blood collected	Serum collection date
UV9	O Manisa	DC	O UKG	–	98	C	174	17/09/03
UV10	O Manisa	DC	O UKG	–	161	C	174	17/09/03
UV11	O Manisa	DC	O UKG	–	161	C	174	17/09/03
UV13	O Manisa	DC	O UKG	–	147	C	174	17/09/03
UV17	O Manisa	DC	O UKG	–	168	C	174	17/09/03
UV19	O Manisa	DC	O UKG	–	147	C	174	17/09/03
UZ58	A Iran 96	I	A Iran 96	–	28	C	32	15/12/03
UZ59	A Iran 96	I	A Iran 96	–	28	C	32	15/12/03
UZ60	A Iran 96	I	A Iran 96	–	28	C	32	15/12/03
UZ62	A Iran 96	I	A Iran 96	–	28	C	32	15/12/03
UY78	O Manisa	DC	O UKG	–	34	C	106	10/03/04
UY83	O Manisa	DC	O UKG	–	91	C	107	11/03/04
UY90	O Manisa	DC	O UKG	–	70	C	106	10/03/04
VE63	Asia 1 Shamir	I	Asia 1 Shamir	–	29	C	42	31/08/04
VE64	Asia 1 Shamir	I	Asia 1 Shamir	–	29	C	42	31/08/04
VE65	Asia 1 Shamir	I	Asia 1 Shamir	FMD	35	C	42	31/08/04
VE66	Asia 1 Shamir	I	Asia 1 Shamir	–	35	C	42	31/08/04
VE67	Asia 1 Shamir	I	Asia 1 Shamir	FMD	29	C	42	31/08/04
UZ68	N/A	I	A Iran 96	FMD	28	C	33	16/12/03
UZ69	N/A	I	A Iran 96	FMD	28	C	33	16/12/03
UY95	N/A	DC	O UKG	FMD	70	C	107	11/03/04
UY96	N/A	DC	O UKG	FMD	50	C	107	11/03/04
UV26	N/A	DC	O UKG	FMD	28	C	174	17/09/03
VH44	N/A	DC	O UKG	FMD	35	C	40	22/12/04
VH45	N/A	DC	O UKG	FMD	35	C	40	22/12/04
VE73	Asia 1 Shamir	I	Asia 1 Shamir	–	–	–	43	01/09/04
VE71	Asia 1 Shamir	I	Asia 1 Shamir	–	22	–	43	01/09/04
UZ54	A Iran 96	I	A Iran 96	FMD	7	–	32	15/12/03
UY80	O Manisa	DC	O UKG	–	21	–	106	10/03/04
UY79	O Manisa	DC	O UKG	–	7	–	106	10/03/04
UV4	O Manisa	DC	O UKG	–	4	–	174	17/09/03
UV15	O Manisa	DC	O UKG	–	4	–	174	17/09/03
UY77	O Manisa	DC	O UKG	–	4	–	106	10/03/04
VE60	N/A	I	Asia 1 Shamir	FMD	8	–	42	31/08/04
VE62	N/A	I	Asia 1 Shamir	FMD	3	–	42	31/08/04
UY94	N/A	DC	O UKG	FMD	10	–	107	11/03/04
UV24	N/A	DC	O UKG	FMD	21	–	174	17/09/03
UV23	N/A	DC	UKG 34/01	FMD	10	–	37	08/05/03

Test results before (grey bars) and after (black bars) heat treatment of sera at 56ºC for 2 hours; a) Cedi test (50% cut-off); b) Bommeli test (20-30% cut-off). Data shown for the12 out of 18 sera from vaccinated cattle that became FMDV carriers after infection and in which percentage positivity in Bommeli test was less 50.

a) Cedi



b) Bommeli



Serology Proficiency – future priorities

- **Proficiency testing**

What is optimal number of distributions per year?
How do we deal with the different species, different serotypes, different purposes of testing and different tests?
What are the priorities?
How much serum is needed?

- **Reference sera**

Establish strong positive bovine serum for each serotype and subtype
Establish bulk negative bovine serum
Establish same for serotype O only from pigs and sheep

- **Vaccine matching serology**

Priority for new network of OIE/FAO reference Laboratories
Will require distribution of bvs and vaccine viruses

Appendix 5

FOOT-AND-MOUTH DISEASE CURRENT SITUATION IN PAKISTAN AND THE REGION

Dr. Manzoor Hussain, Regional Epidemiologist, GTFS/INT/907/ITA

Regional Workshop on Progressive Control Strategies for Foot and Mouth Disease, Bhur Ban Murree Pakistan, (September 11-13, 2004)

Recommendations

A. Regional Approaches

- Diseases like FMD can be controlled only if there is a strong regional cooperation and an integrated regional approach keeping in view the nature of the disease and the movement of animals in different countries.

- We strongly support SAARC-GF-TADs Regional initiative that consists of components like regional support unit, regional reference labs, epidemiological network and centre and technical backstopping by OIE and FAO. We recommend that OIE be actively involved in this process and assist in ensuring a strong focus on FMD similar to that undertaken for the SEAFMD model.

- We strongly recommend that GF-TAD also take an initiative similar to the one in SAARC in the central Asian Countries and should include Pakistan, Afghanistan and Iran in this initiative. Italian-FAO project, EU-Pakistan Project and EU-Afghanistan project should develop an overlapping coordination mechanism in this regard. These projects can immediately start sharing the information on epidemiology and control of the TADs in the participating countries.

B. National Approaches

- Both public and private sectors should be involved in design and implementation of strategies.
- Valid national FMD control strategy will require comprehensive epidemiological information of the disease in each country.

- Effective disease reporting, early warning and rapid response mechanism become progressively more important for effective FMD disease control strategy.

- Availability of relevant and effective vaccine is an important tool for the control of FMD in the region. Cold chain system for vaccine needs to be ensured and country should have a national quality control mechanism other than the manufacturer of the vaccine.

- Diagnostic laboratories and research institutions be appropriately strengthened.
- Awareness and capacity building of different stake-holders for implementation of FMD control should be focused in the strategy.

- There should be a written national policy document for each country which should include emergency preparedness program in case of outbreak. This policy document should be notified.

- The control strategy should emphasize areas which can show early visible benefits to make it more saleable to the policy makers. For Pakistan, the control strategy should first address commercial farming (urban and peri-urban dairying), followed by market-oriented small holders and finally subsistence farmers. Commercial farmers will be willing to share cost for the control strategy and will be more responsive to the efforts being made in this regard.

Regional Project, "Controlling Transboundary Animal Diseases in Central Asian Countries"

- To progress the verification of freedom from Rinderpest
- To better understand the impact of PPR, FMD and other important diseases in the region
- To establish communication between the countries for collaborative disease control
- To establish national disease investigation, control and contingency planning for TADs

FMD in Afghanistan

- The disease present throughout the year
- Surveillance system not efficient
- Minimal diagnostic facilities
- Serotypes A, O and Asia 1 prevalent
- No facility for vaccine production (only imported trivalent vaccine used when available)

FMD in Uzbekistan

- Last laboratory confirmed FMD case (serotype A) in 1991 (year of independence)

- Serotypes A, O reported in past
- Vaccination (6 million) being carried out in bordering areas (Tajikistan, Afghanistan, Kazakhstan)
- Surveillance and diagnostic facilities not satisfactory

FMD in Tajikistan

- Last case reported in 2004 (Asia 1)
- Serotypes A, O, Asia 1 prevalent
- Vaccination from Russia being used in bordering Afghanistan area
- No facilities for confirmation of the disease and typing virus serotypes
- Surveillance system not efficient

FMD in Turkmenistan

- Last case of FMD reported in 2000
- Vaccination in buffer zones (bordering Kazakhstan, Afghanistan, Uzbekistan, Iran)
- Minimal facilities for Lab confirmation
- Veterinary Department being run on self-supporting basis since 1998

FMD in Pakistan

- Every year, outbreaks reported throughout the country
- Serotypes A, O, Asia 1 prevalent
- FMD Referral Lab recently established
- Local vaccine monovalent (O type) and quantity insufficient (0.5-0.8 m doses)
- Dairy colonies, the main source of virus reservoir and transmission
- Severity of the disease increased during last two years

Administrative divisions

- 4 Provinces (Punjab, Sindh, Balochistan, and NWFP),
- One state (AJK), and
- One Capital Territory (Islamabad Capital Territory)
- One Territory (Federally Administered Tribal Areas)

Area

- **Land boundaries:**
  Total: 6,774 km

  B*order countries:*

- Afghanistan 2,430 km
- China 523 km
- India 2,912 km
- Iran 909 km

Livestock in national economy

- Agriculture in Pak GDP 23.6 %
- Livestock in Pak GDP 11.4 %

- Share in agri GDP 49.1 %
- Livestock in export 8.5 % (935 m US$)
- Provides raw material for industry
- Creates market and capital
- Social security for rural poor
- Security against crop failure in barani areas
- Dependent population > 6.5 m families

Economic Survey (2003-04)

LIVESTOCK POPULATION (2003-04)

(Million Heads)

PROVINCE	CATTLE	BUFFALO	SHEEP	GOAT	CAMEL
PAKISTAN	**23.8**	**25.5**	**24.7**	**54.7**	**0.8**
	Per cent distribution				
NWFP	**21.5**	**6.3**	**13.3**	**17.5**	**8.3**
PUNJAB	**43.2**	**60.8**	**24.3**	**37.1**	**18.6**
SINDH	**28.9**	**31.8**	**18.2**	**23.8**	**29.7**
BALOCH-ISTAN	**6.4**	**1.1**	**44.2**	**21.6**	**43.4**

Economic Survey (2003-04)

LIVESTOCK PRODUCTION

- **Milk 28.624 M ton**
- **Beef 1.087 M ton**
- **Mutton 0.723 M ton**
- **Poultry meat 0.402 M ton**
- **Eggs 8.247 billion**
- **Wool 39.7 T ton**
- **Hair 19.9 T ton**
- **Skins and hides 48.5 millions**

Economic Survey (2003-04)

Governance of Veterinary Service in Pakistan

- Federal Government
  - Policy, International and Provincial Coordination, Quality Control, R & D in critical areas and Animal Quarantine, Import & Export
- Provincial Governments *(Punjab, Sindh, NWFP, Balochistan, Northern Areas, Azad Jammu & Kashmir)*
  - Provincial policy, Livestock Research & Development, Diagnostic Laboratories and Livestock Extension activities
- District Governments
  - Veterinary Hospitals and Dispensaries

Disease	Status (# cases)
Rinderpest	**Nil**
Contagious bovine pleuropneumonia	**Never reported**
Bovine spongiform encephalopathy	**Never reported**
Foot & Mouth Disease	**+**
Peste de Petits Ruminants	**4360**
Sheep/goat Pox	**52001**
Haemorrhagic Septicemia	**53535**
Blackquarter	**29404**
Contagious caprine pleuropnuemonia	**14094**
Enterotoxaemia	**7895**

Livestock Disease Status of Pakistan 2004

Rinderpest in Pakistan

- Status
  - Provisional freedom (January 2003)
  - Last case (Sept 2000), No vaccination since Nov 2000
  - Freedom from disease aimed in 2006-7
- Diagnosis
  - Agar gel diffusion
  - ELISA (both antigen & antibody)
  - PCR
  - Virus isolation
- Control
  - Disease search through PDS & Sero-surveillance
  - Vaccine manufacturing facility (1) and Banks (3)
  - Contingency plan in place

Foot & Mouth Disease in Pakistan

- Status
  - Disease is endemic and sporadic in occurrence
  - Severe disease in crossbred & exotic animals
  - Disease is also important in high producing animals
  - Serotypes A, O and Asia 1 with "O" being more prevalent
  - Serotype C reported only once in 1960s
  - The most prevalent disease as per PDS reports
- Diagnosis
  - Complement fixation test
  - ELISA (both antigen & antibody)

  - PCR (under development)
  - Virus isolation (facilities not yet developed)
- Control
  - Notifiable disease
  - Progressive control of disease
  - Vaccine manufacturing facility – limited capacity & quality

Peste de Petits Ruminants in Pakistan

- Status
  - First reported in 1990
  - Is a new emerging disease
  - Sporadic occurrence in selective areas
  - Epidemiology study is under way
- Diagnosis
  - Agar gel diffusion
  - ELISA (both antigen & antibody)
  - PCR (under development)
- Control
  - Current Strategy is disease control
  - Vaccine manufacturing facility (FAO project)
  - Imported vaccine (under FAO project)

- Commercial Dairy Colonies Karachi

- Landhi Dairy Colonies 200,000
- Bilal Dairy Colony 11,000
- Al-Momin Dairy Society 13,000
- Nagory dairy Society 10,000
- Baldia Town 150,000
- Total Dairy Animals in Karachi: 0.8 million

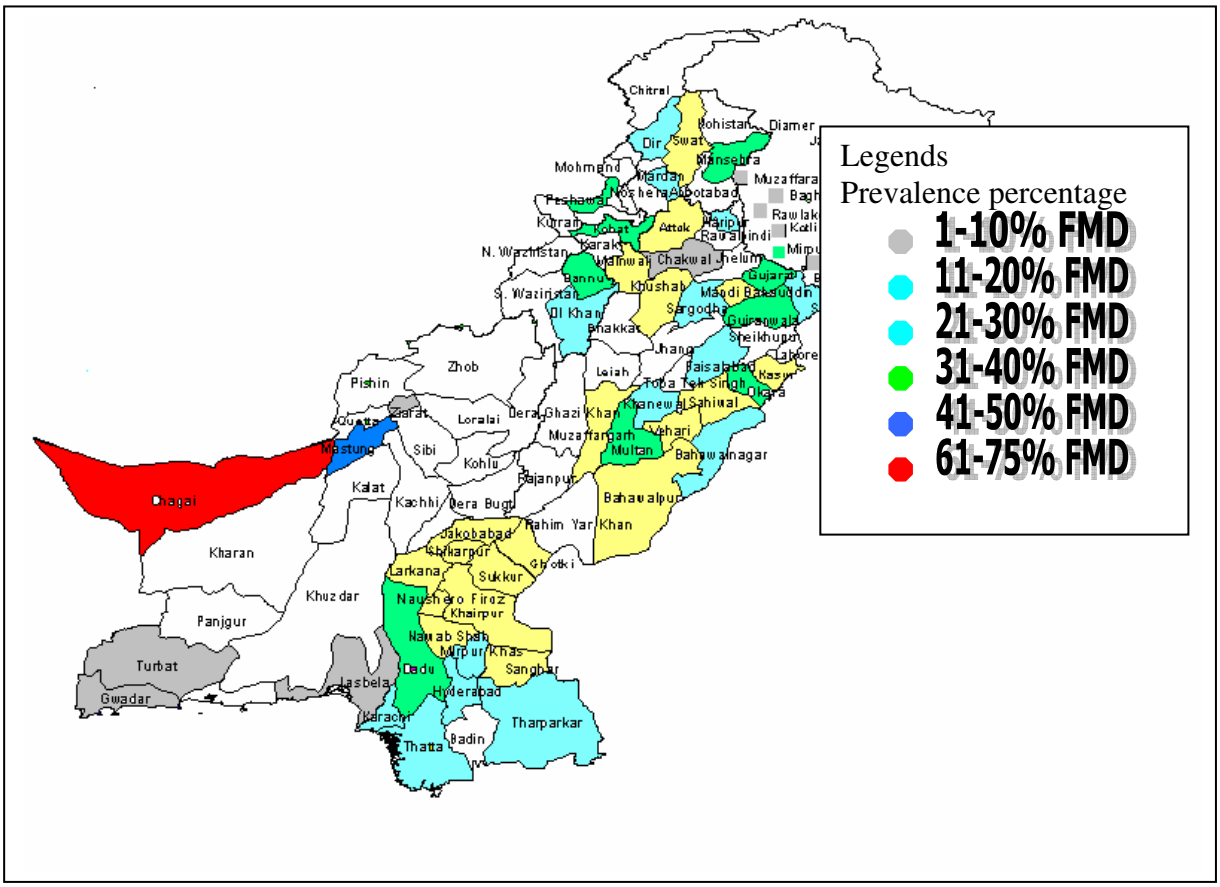
Vaccine Production in the Country

- Vaccines manufactured in both public and private sector (only one for FMD)
- 5 Public sector set-ups
- 5 Private sectors set-ups
- Poultry vaccines also

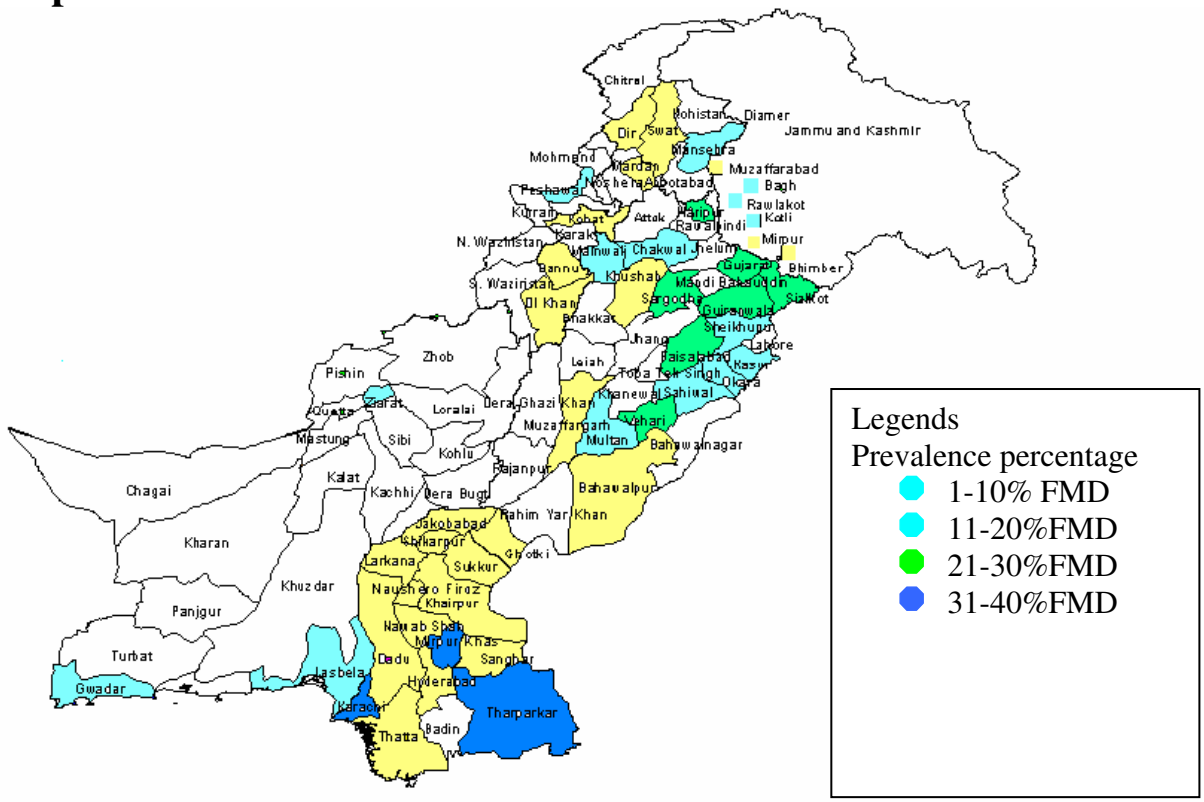
Priorities in Disease Control

- Control and eradication of diseases of trade and economic importance
  - Freedom from Rinderpest
  - Improved legal framework
  - Control of PPR, FMD
  - Enhanced coverage through vaccination against endemic diseases
- Strengthening of disease diagnostic service
- Capacity building of all stakeholders

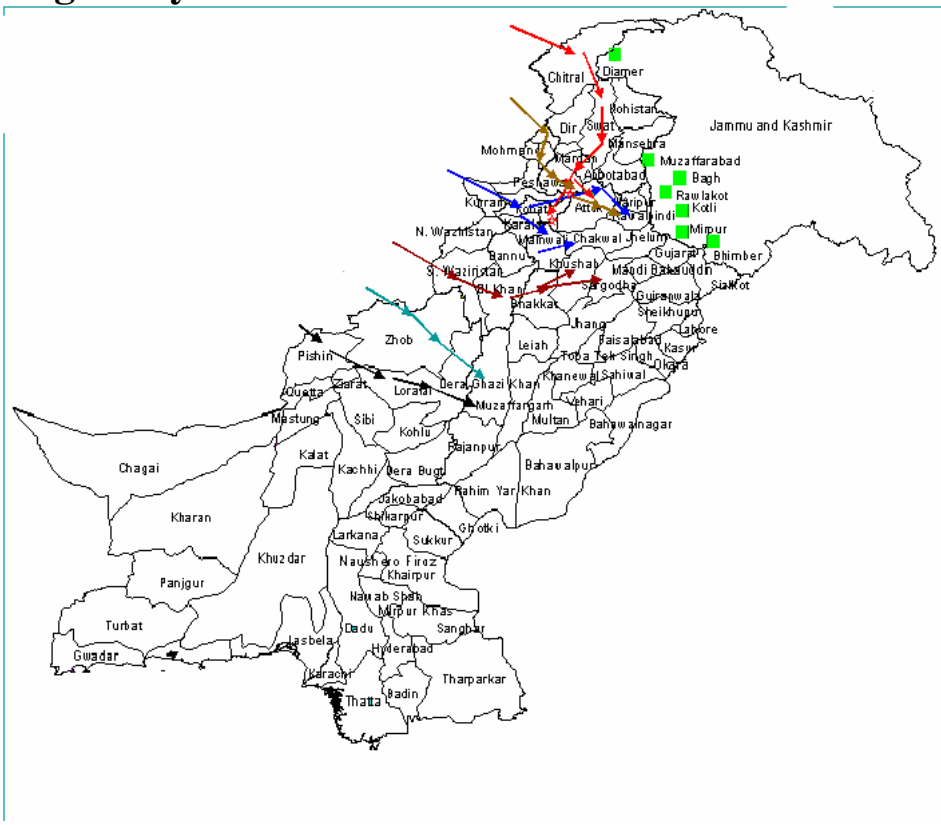
Prevalence of FMD in Pakistan 2000-2004

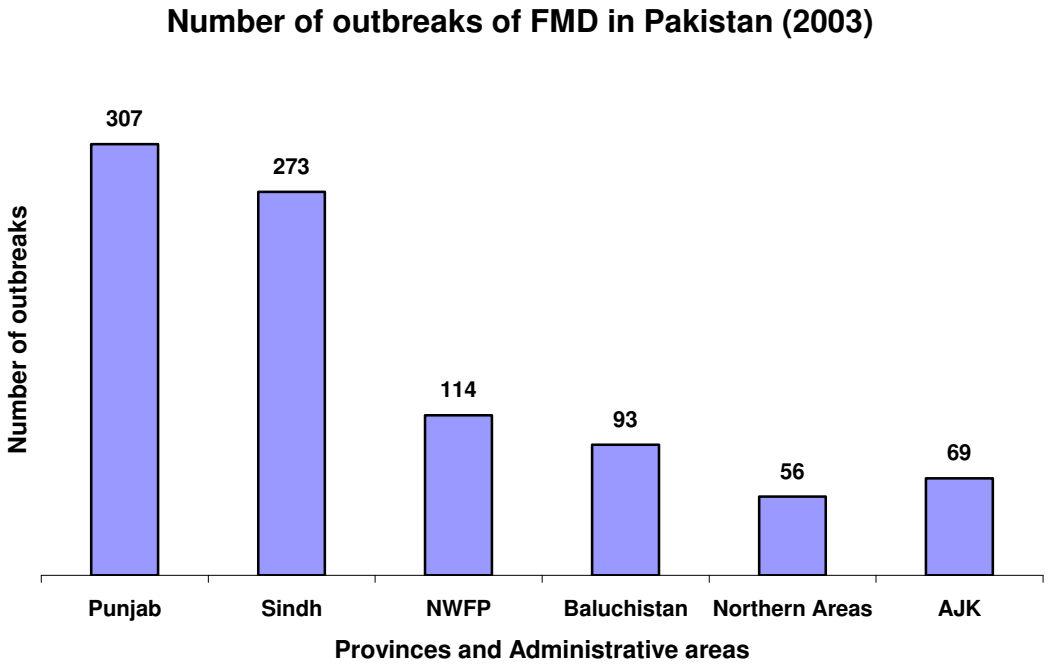


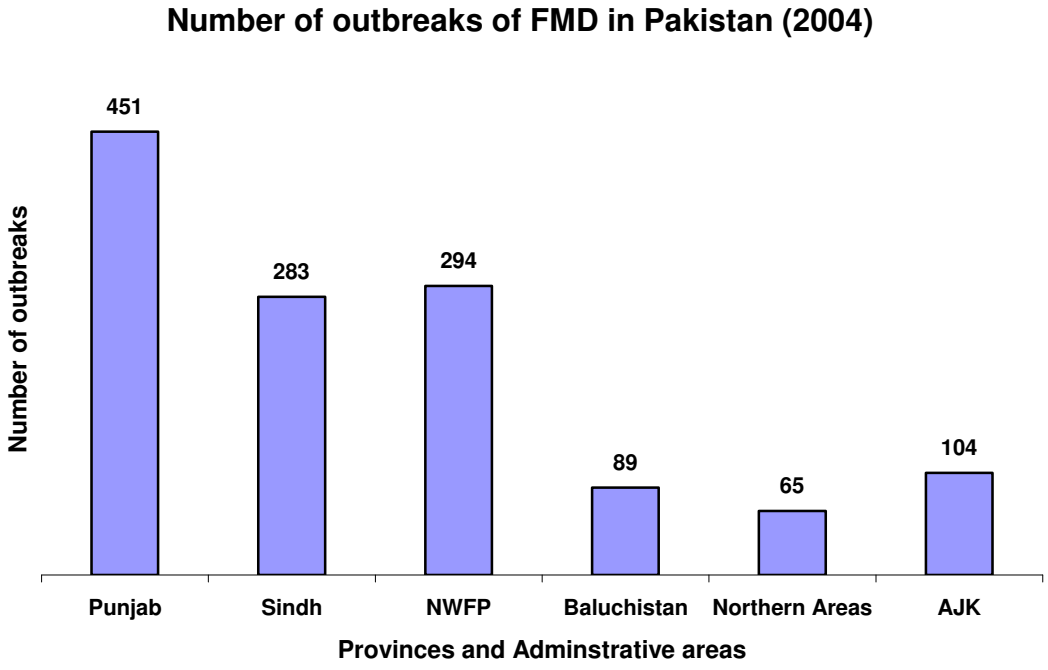
Importance of FMD in Pakistan 2000to 2004



Migratory routes of cattle in Pakistan 2000 to 2004




Number of outbreaks of FMD in Pakistan (2003)
307
273
114
93
56
69
Number of outbreaks
Punjab
Sindh
NWFP
Baluchistan
Northern Areas
AJK
Provinces and Administrative areas


Number of outbreaks of FMD in Pakistan (2004)
451
283
294
89
65
104
Number of outbreaks
Punjab
Sindh
NWFP
Baluchistan
Northern Areas
AJK
Provinces and Adminstrative areas

FMD virus sero-types In Pakistan
(August 2003 – August 2005)

Province/ Area	Total samples submitted	Total positive	O	A	Asia 1	Improper/not tested
Punjab	**109**	**36***	**27**	**1**	**9**	**-**
Sindh	**100**	**9**	**8**	**1**	**-**	**6**
NWFP	**78**	**15***	**8**	**2**	**6**	**9**
Balochistan	**20**	**13**	**3**	**9**	**1**	**-**
ICT	**31**	**5**	**5**	**-**	**-**	**2**
NA	**21**	**14**	**14**	**-**	**1**	**1**
Total	**359**	**92**	**65**	**13**	**17**	**18**

RECOMMENDATIONS

Diagnostic facilities

There is a need to establish appropriate facilities for FMD diagnosis and confirmation of virus serotype(s) in the region. ELISA equipment/kits and training is being provided by the Regional Project in all beneficiary countries. It should be supported for further capacity building by the EU Projects in Pakistan and Afghanistan

- **Surveillance**
  FMD virus serotype(s) prevalent in the region should be monitored on sustainable basis. The samples should also be sent to the WRL for further analysis.
  The Regional Project will be able to generate and share this information mainly for the beneficiary countries.

- There is considerable movement of animals between Pakistan, Afghanistan and Iran. There is a need that policy makers/scientists from these countries meet on regular basis and based upon the existing knowledge, develop a strategy for the control of FMD in the region

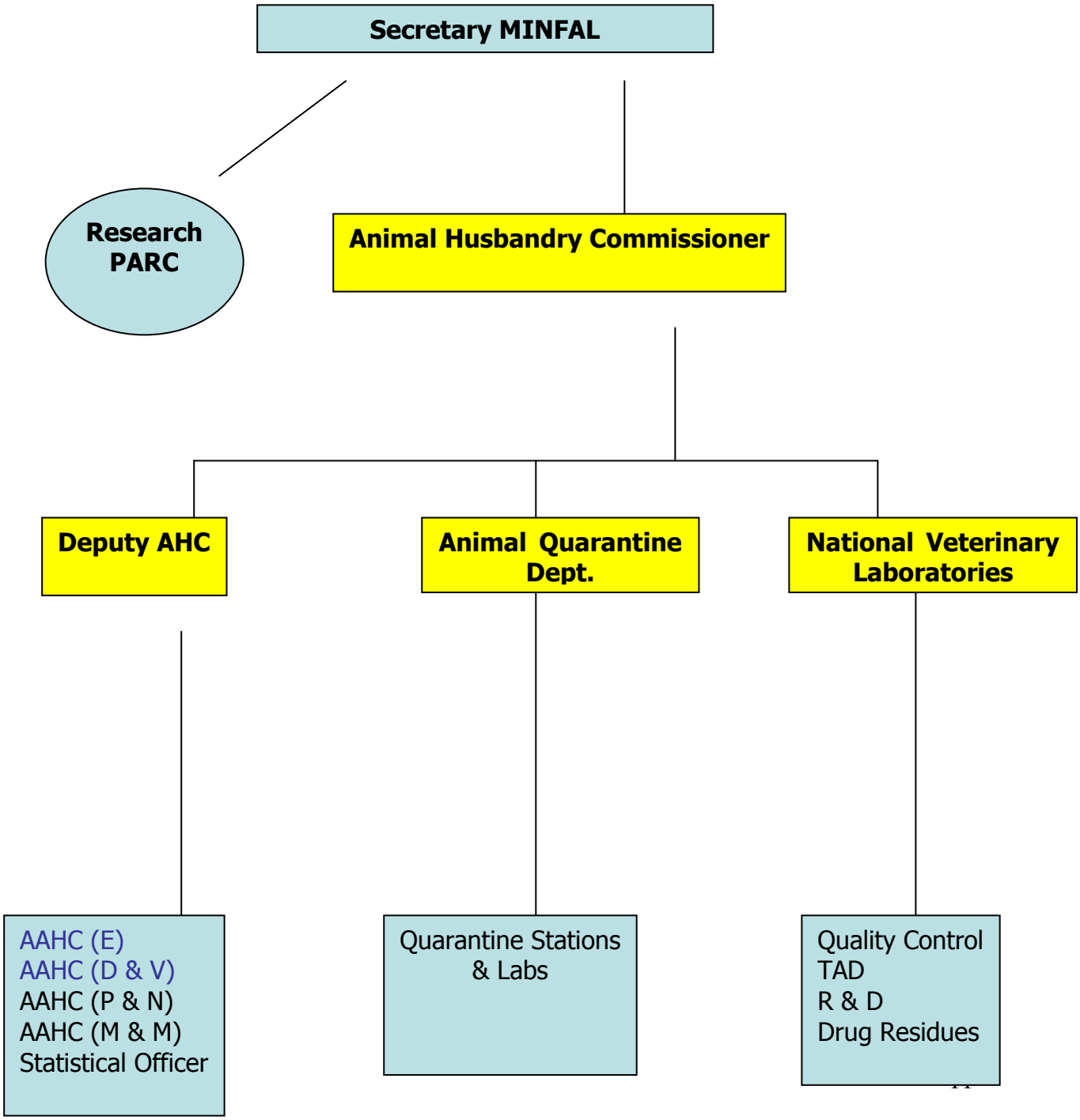
- **Epidemiological Analysis**
  Very little information is available about the epidemiology of FMD in the region. Efforts are being made by the Regional Project to yield this data in 5 beneficiary countries. However, it is important that this activity is supported and information shared by the countries like Iran, Kyrgyzstan and Kazakhstan to develop a better approach for the control of FMD in the region.

- **FMD Vaccine Production**
  Quality assured FMD vaccine containing appropriate virus serotype(s) is not produced in the region. Only commercial dairy farmers are buying this vaccine from other multinational companies

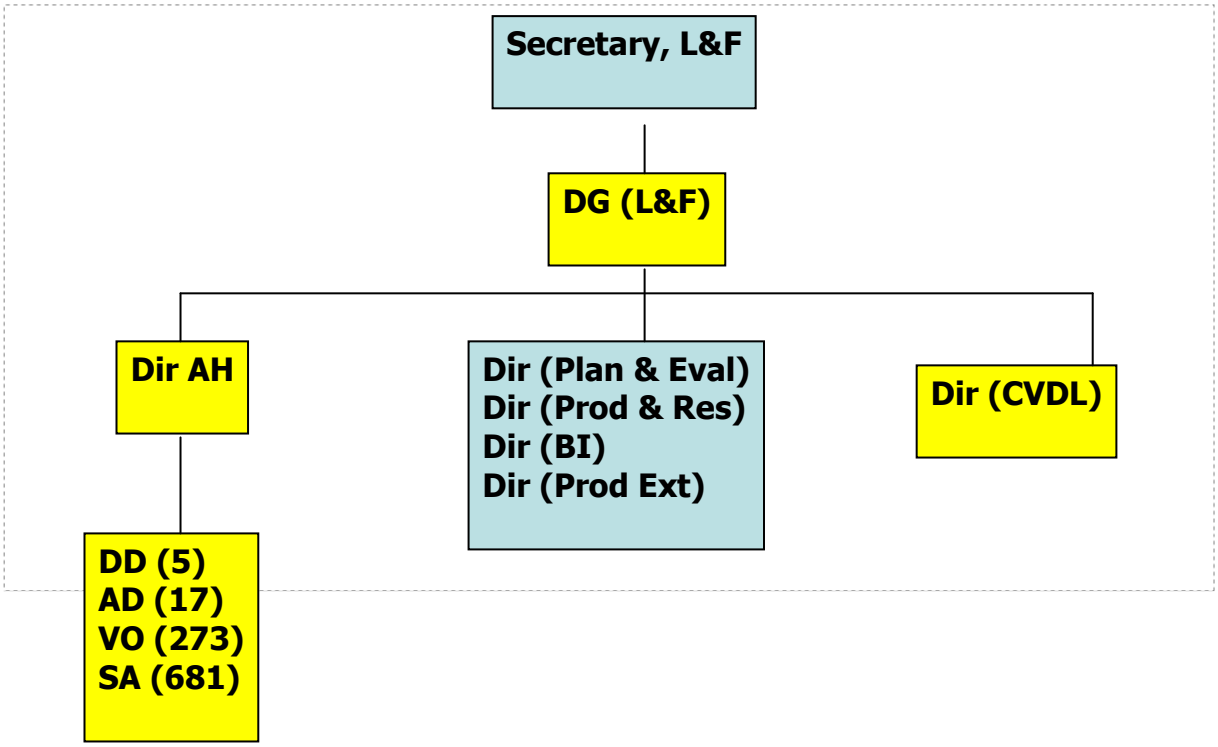
on a higher price. It is recommended that facilities should be established for the production of quality FMD vaccine at a suitable place in the region.

- Training:
  - Quality testing of vaccines
  - Virus isolation and identification
  - Strengthening of FMD Referral Lab
  - Application of FMD pen-side test??

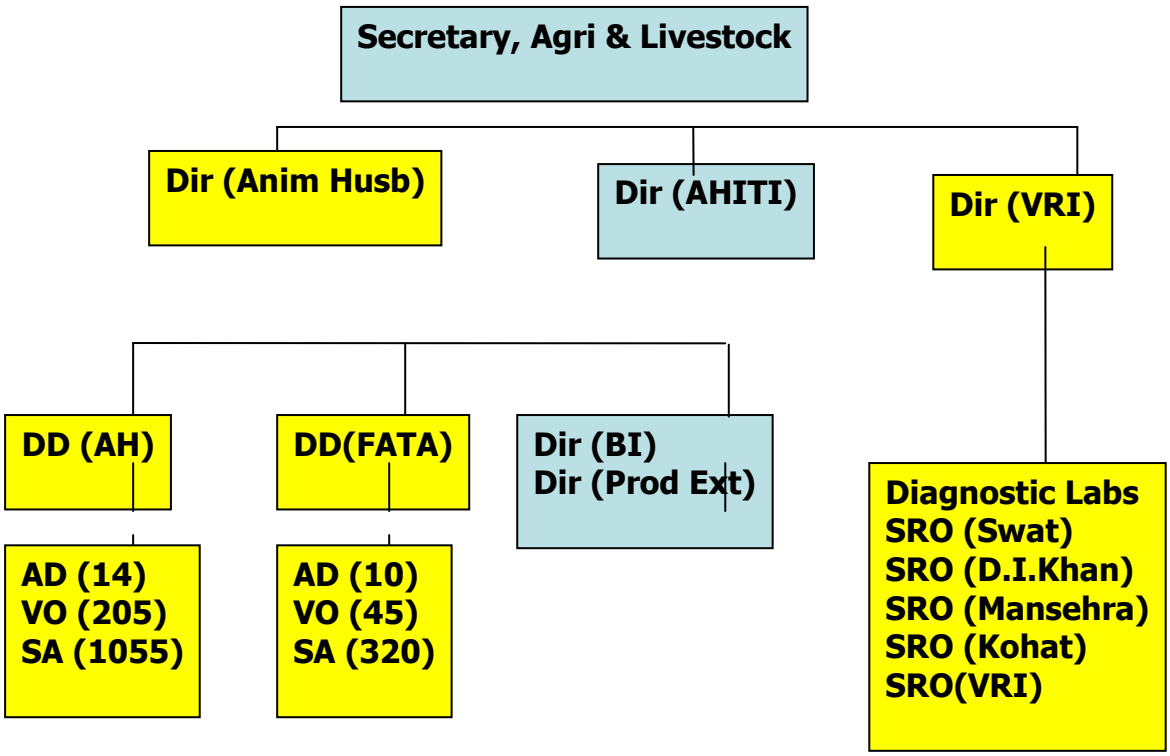
Veterinary Service in Federal Government



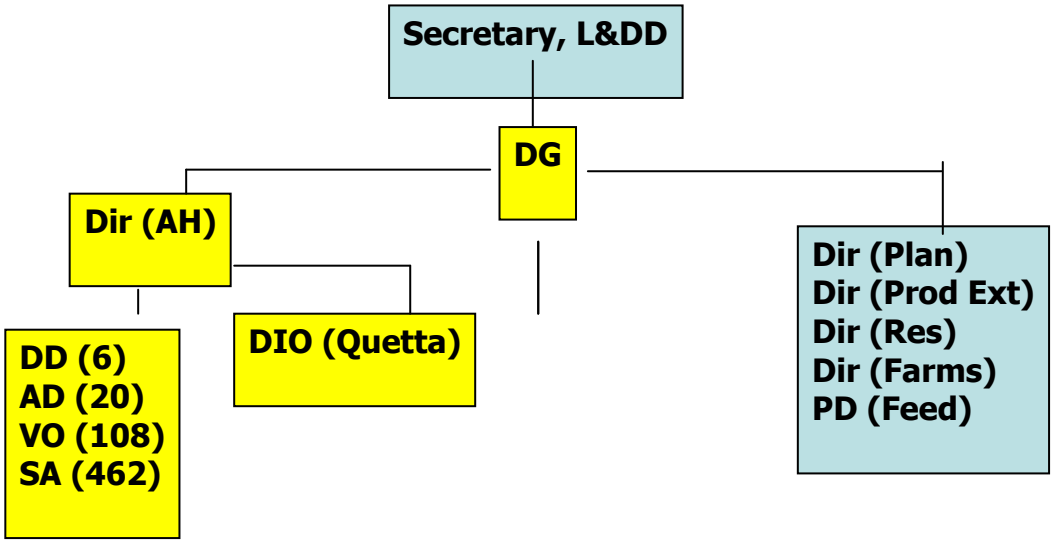
Veterinary Service in Sindh



Veterinary Service in NWFP



Veterinary Service in Balochistan



Appendix 10

Evaluation report for the organization of FAO collaborative studies (Phase XIX) 2005/2006 - needs and desires of the NR FMD laboratories for FMD antigen and antibody detection and development of proficiency inter-laboratory and exercises for QA tests for the neighbouring EU countries

Assoc. Professor Dr. Georgi Georgiev (PhD), Bulgaria

The countries involved in the questionnaire were: From the Western Balkan countries: The FYR of Macedonia, Albania, Bosnia and Herzegovina, Serbia and Montenegro, Croatia. From The Former Soviet Union Countries: Ukraine, Moldova, Belarusia. From the Transcaucasus countries: Georgia, Armenia, Azerbaijan.

The aim of this report is to try to summarize the needs and readiness of FMD NRL of the countries for their accreditation and participation in ring tests on FMD virus and antibody detection.

Background:

On the previous FMD proficiency (Phase 16, 17 and 18) collaborative studies for FMD virus and antibodies detection the results were assessed on the Open Sessions of the FMD RS Group in Borovetz, Bulgaria - 2000, Izmir, Turkey - 2002 and China, Crete, Greece - 2004.
In Phase XVI – year 2000, of the 33 member countries of the EUFMD Commission 23 of them participated. From Western Balkan countries Albania, Serbia, Croatia and The FYR of Macedonia were requested to participate. The results from Phase XVI collaborative exercise were submitted only from The FYR of Macedonia and Serbia. LPBL ELISA evaluation results were submitted for O, A and C Serotypes. VNT was not done, FMD Ag evaluation was not included in the exercise.

The Former Soviet Union and Transcaucasus countries were not requested and did not participate.

Phase XVII was carried out after the great FMD outbreak and epidemic in Western Europe in 2001 and only 9 out 33 countries participated.
The Results from Phase XVIII were summarized, assessed and reported at the Open EUFMD Research Group Meeting in Chania, Crete, Greece in 2004. For the first time test sera with FMD NSP antibodies were included. Neither Western Balkan countries, nor Former Soviet Union or Transcaucasus countries participated.

The questionnaire was provided with the following:

1. Does your FMD NRL participate in ring proficiency tests for FMD antigen evaluation using Ag ELISA or RT-PCR?
2. Does your FMD NRL participate in ring proficiency tests for FMD antibody evaluation – LPBL ELISA or SPCE ELISA?
3. Does your FMD NRL participate in ring proficiency tests for NSP FMD antibody evaluation – NSP ELISA?
4. Does your FMD NRL develop the QA&QC rules and what is your accreditation strategy in the next few years?

In the questionnaire the following FMD NRL were involved:

Western Balkan countries: FYR of Macedonia, Albania, Bosnia and Herzegovina, Serbia and Montenegro, Croatia.

Former Soviet Union Countries: Ukraine, Moldova, Belarusia.

Transcaucasus countries: Georgia, Armenia, Azerbaijan.

Results:

FMD NRL of Albania contact persons: Dr. Zace Malaj, Director General of Veterinary Service of Albania and Dr. Aldin Lika – Head of the FMD NRL.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	no	**no**	**no**	**no**	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	**yes**		**yes**	

1. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.

2. Positive answer for developing accreditation strategy and EQ&A.

FMD NRL of FIROM Serbia and Montenegro contact persons: Dr. Dejan Krnjaik, CVO Serbia and Montenegro; Dr. Bosiljka Djuricic, Professor, Belgrade University;
Dr. Bosiljka Djuricic, Professor, Belgrade University.

Now Serbia and Montenegro veterinary service has created a new laboratory complex with all facilities to work with high contingency infections including FMD.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	no	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	yes		yes	

1. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.
2. Positive answer for developing accreditation strategy and EQ&A.
3. Supporting in ELISA kit tests delivering.

FMD NRL of Croatia contact persons: Dr. Mate Brstilo, CVO; Dr. Sanja Separovic, Head Animal Health Department, Croatia.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	yes	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	yes	no	no	

1. They perform NSP ELISA only for research purposes, but not routinely.
2. Positive answer for participation in a ring test for structural and NS antibody ELISA evaluation.
3. Positive answer for developing accreditation strategy and EQ&A.
4. Need for supporting of ELISA kit tests.

FMD NRL of Bosnia and Herzegovina **c**ontact person: Dr. Jozo Bagaric, Director State Veterinary Administration of Bosnia and Herzegovina.

No Response received!

FMD NRL of The FYR of Macedonia contact person: Dr. Sloboden Cokrevski, CVO FYR of Macedonia; Dr. Ivancho Naletvoski, Head of laboratory Virus Diseases of Ruminants Veterinary Faculty, Skopje, FYR of Macedonia.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	yes	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	yes	no	yes	

1. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.
1. Positive answer for participation in a ring tests for FMD Ag ELISA evaluation.
2. Positive answer for developing accreditation strategy and EQ&A.
3. Need for supporting of ELISA kit tests.

FMD NRL of Ukraine contact person: Dr. Petro Ivanovitch Verbitski, Head of State Department of Veterinary Medicine, Ukraine.

Contact wad made. No Response received!

FMD NRL of Moldova contact person: Dr. Efim Renita, Chief of the Department of Veterinary Medicine, Moldova Republic.

The contact was not available.

FMD NRL of Belarus contact person: Dr. Aleksandr Aksenov, Head of Veterinary Service of Belarus.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	no	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	no	no	yes	

1. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.
2. FMD NRL is accredited by ISO-17025
3. Positive answer for participation in EQ&A.
4. Need for supporting of ELISA kit tests.

FMD NRL of Armenia contact person: Dr. Grigori Baghian, Head State Veterinary Service, Armenia.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	no	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	no	no	yes	

1. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.
2. Need for supporting of ELISA kit tests.

FMD NRL of Azerbaijan contact person: Dr. Tamilla Alyeva, The National Consultant for project TCP/RER/3001, Azerbaijan.

What the FMD NRL can perform?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	no	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	no	no	yes	

1. Positive answer for participation in a ring tests for FMD Ag ELISA determination.
2. Positive answer for participation in a ring tests for structural and NS antibody ELISA evaluation.
3. Positive answer for developing accreditation strategy and EQ&A.
4. Need for supporting of ELISA kit tests.

FMD NRL of Georgia contact persons: Dr. Levan Ramishvili, Councill of the Ministry of Agriculture, Georgia; Dr. Jambul Malahelidze, Head Department of Veterinary, Ministry of Agriculture of Georgia.

The creation of a new Georgian FMD NRL relating to the American contract "Biological Weapons Proliferation Prevention" is going on. They declare that the laboratory reconstruction will finish of the end of October this year. After this date they will be able to start to work.

What the FMD NRL can perform after refurbishing?

Tests for Ag	**Ag ELISA**	**RT-PCR**	**VNT**	**Isolation**	
	yes	yes	no	no	
Tests for Ab	**LPBL ELISA**	**SPCE ELISA**	**VNT**	**NSP ELISA**	
	yes	no	no	yes	

No wishes and needs in the letter.

Conclusions:

On the basis of the answers from the questionnaire we can summarize the following:

1. Most FMD NRLs can perform the basic serological tests for FMD Ab ELISA determination.
2. Only 7 out of 11 of the NRL declared that they can perform FMD Ag ELISA.
3. Only 1 out of 11 of the NRL declared that it has accreditation ISO-17025.
4. The most of NRL need for delivering of diagnostic kits.
5. No NRL requests for training of the staff.
6. No NRL requests for equipment support.
7. All NRL positive answered for participation in a ring tests for structural and NS antibody ELISA evaluation.
8. All NRL positive answered for developing accreditation strategy and EQ&A.

Activities of OIE and EU Coordinated Action on FMD/CSF

David Paton & Christianne Bruschke

EU Coordinated Action on FMD-CSF

Jan 2005 – Dec 2007

- FMD part coordinated by IAH-Pirbright
- CSF part coordinated by TIHO-Hannover
- 7 other European partner laboratories
- OIE and FAO participation
- Bring together expertise and encourage collaboration
- Analyse what is currently done – strengths, weaknesses, gaps
- Establish new networks and collaborative agreements

OIE Ad Hoc Group on FMD Antigen and Vaccine Banks

- Two meetings in June 2004 and April 2005
- New texts agreed for OIE Manual
  - New chapter on antigen and vaccine banks
  - Revised section in existing FMD chapter on FMD vaccines
  - New section in existing FMD chapter on FMD vaccine matching tests
- Review paper submitted to OIE Sci et Tech Rev. on Selection of vaccine strains

OIE/CA Initiative on Vaccines and Antigen Banks

- Problem of responding to surges in vaccine demand
- Many national and regional FMD vaccine banks established
- Many common issues and duplicated effort to address these
- Develop a network of vaccine bank managers
- Work towards common standards of vaccine production and certification
  - New sections for OIE Manual on vaccine reserves and on vaccine selection
- Ultimate aim is a global FMD vaccine reserve
- Present aim is to find out who are the key players and see if they want to cooperate with one another

New OIE/FAO network of FMD reference laboratories

- Participants are the OIE/FAO reference laboratories in UK, Brazil, Botswana and Russia
- Secretariat at IAH-Pirbright and FAO/OIE steering group
- To gather, generate and make available laboratory information on the global occurrence and spread of FMD
  - Improve interaction and co-operation between reference Laboratories
  - Clarify mandates
  - Agree procedures for exchange of materials including viruses
  - Develop equivalence in testing – especially vaccine matching
  - Develop common systems for providing and sharing information in real-time

OIE Ad hoc group on NSP tests

- Next meeting is planned on January 23-25
- Aim to validate NSP data on pigs and sheep
  - Sheep data to be provided by Ingrid Bergmann
  - Pig data to be provided from Taiwan

Post Vaccination Surveillance

- Issue of whether or not current guidelines meet European requirements
  - Emergency vaccination and return to status of *free without vaccination*
  - Use of NSP serosurveillance to *Indicate* or *substantiate* rather than demonstrate *freedom from infection*
- No changes have been made
- Specific request needed from a member country

New two year cycle for document approval

- Request made to OIE by member country
- Considered by relevant commission and if necessary referred to expert group
- Document agreed by relevant commission in January and sent to member countries for comment
- Comments reviewed in next year and finalised by relevant commission following January
- Documents agreed at May General Assembly

International Regulations for transport of infectious substances, including diagnostic samples

- UN Sub-committee on Transport of Dangerous Goods
  - All national and international transportation
  - New rules January 2005
  - OIE proposals on diagnostic specimens incorporated into an amendment effective for air transport in March 2005
- Category A infectious substances
  - On exposure, cause permanent disability, life threatening or fatal disease in otherwise healthy humans or animals
  - Includes cultures of FMDV (UN2900 Cat A animal pathogen)
- Category B infectious substances
  - Non category A cultures
  - Diagnostic tissue samples for all diseases except CCHF, Nipah, monkeypox
  - Slightly less stringent packaging & much less restrictive shipping

DIAGNOSTIC PERFORMANCE OF NSP TESTS

NRL for FMD

Istituto Zooprofilattico Sperimentale

Brescia (Italy)

IZSLER, Brescia, Italy
PANAFTOSA, Rio, Brasil
CODA-VAR, Ukkel, Belgium
CIDC-Lelystad,The Netherlands
IAH, Pirbright, UK
KVI, Bet Dagan, Israel
FLI, Riems, Germany
SAP, Ankara, Turkey
DFVF, Lindholm, Denmark
FAO, Rome, Italy

Objective:

Comparative evaluation of tests available in Europe for the detection of Ab to NSP

A consortium of laboratories within the research group, was established to answer these questions

In Europe:

- Which NSP tests are available in Europe?
- What is their validation status?
- How their performances compare with those of the OIE index test?

Approach: collection of well defined experimental and field sera and testing in parallel with the tests under evaluation

Brescia workshop; 3 – 15 May 2004

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Analysis of results

Volume of data is adequate to validate the tests for use in cattle

Analyses performed for cattle

- ✓ Diagnostic Specificity
- ✓ Diagnostic Sensitivity
- ✓ Analysis of correlation between tests (specificity § sensitivity covariances)
- ✓ Analytical Sensitivity
- ✓ Rate of NSP antibody seroprevalence detected in post outbreaks surveys
- ✓ Analysis of concordance/discrepancy (field samples)

More samples are needed to establish sensitivity in sheep and pigs - Preliminary data on comparative Sp and Se based on limited sets of sera available

Collection of well defined cattle sera

≈ 2500 sera, field and experimental animals, contributed by 9 countries (Belgium, Denmark, Germany, Israel, Italy, The Netherlands, Turkey, United Kingdom, Zimbabwe) and South America

CATTLE condition	NON INFECTED		INFECTED				Post Outbreak ISRAEL ZIMBABWE	Total
	Non Vacc	Vacc	Non Vacc		Vaccinated			
			C+	C-/?	C+	C-/?		
Total sera	675 [F]	425	21	41	225	325	867 [F]	2579
Total cattle	675	425	13	41	67	218	867	2306

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Diagnostic Specificity - RESULTS

1100 cattle sera → 675 naïve, from 4 EU countries
→ 425 vaccinated with European vaccines

Test	UBI	SVANOVA	CEDI	BOMMELI	BRESCIA	PANAFTO
screening	**98.5%**	**98.5%**	**98.1%**	**97.6%**	**97.4%**	**97.2%**
Retest pos	**99%**	**99%**	**99.2%**	**98.8%**	**99.7%**	**98.1%**

- **No significant differences between vaccinated and non-vaccinated populations were found with any of the 6 NSP-ELISA: Vaccination with European vaccines does not affect specificity**
- **Specificity ranges from 97.2% to 98.5% in the different ELISA, at the first screening test**
- **Specificity improves significantly after retesting false-positives, overcoming 99% with the more specific tests**

Diagnostic Specificity – RESULTS (2)

Two further observations

- **False-positives exhibit usually bordeline values in all NSP-ELISA**
- **Specificity covariance: false-positive results are usually not correlated (a serum reacting as false-positive in one test is usually negative in all other tests – none coincident positive result in more than é tests)**

These observations are "strategic" and can be considered when interpreting results of NSP-assays and when designing a testing system

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NSP tests performance
Diagnostic Sensitivity

- Evaluated using experimentally infected cattle
- 612 sera from 322 cattle (some sequential bleedings)
- Animals categorised according:
  - time after infection (7 dpi → > 100 dpi)
  - vaccinated or not vaccinated
  - evidence of infection (VI, PCR, ...)
  - persistent infection (carriers)

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Diagnostic Sensitivity - RESULTS

NON VACCINATED CATTLE EXPOSED TO INFECTION (n° 54)

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
7 - 14	5	100%	100%	100%	100%	100%	100%	1,000
15 - 27	27	100%	100%	100%	100%	100%	100%	1,000
28 - 100	26	100%	100%	100%	96.2%	92.3%	100%	0,301
> 100	2	100%	100%	50%	50%	50%	50%	0,486

VACCINATED CATTLE EXPOSED TO INFECTION (n° 285)

ALL , WITH OR WITHOUT EVIDENCE OF INFECTION

7 - 14	176-177	**49.7%**	**53.1%**	**49.7%**	41.8%	**51.1%**	32.8%	**0.002**
15 - 27	131	**60.3%**	**55.7%**	**52.7%**	49.6%	**52.7%**	38.2%	**0.012**
28 - 100	108	**69.4%**	**64.8%**	**63%**	58.3%	50%	55.6%	0.052
> 100	47	**72.3%**	**63.8%**	**74.5%**	57.4%	38.3%	46.8%	**0.001**

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Diagnostic Sensitivity - RESULTS

VACCINATED CATTLE EXPOSED TO INFECTION
with evidence of infection (n° 164)

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
ONLY CARRIERS (CATTLE KNOWN TO BE PERSISTENTLY INFECTED AT 28 DAYS OR LATER, n° 67)								
7 - 14	29	58.6%	58.6%	58.6%	55.2%	58.6%	41.4%	0,531
15 - 27	36	72.2%	66.7%	63.9%	55.6%	58.3%	58.3%	0,483
28 - 100	**66** [a)]	**93.9%**	**86.4%**	**86.4%**	**71.2%**	**68.2%**	**77.3%**	**0.001**
> 100	37 [a)]	**89.2%**	**78.4%**	**89.2%**	70.3%	48.6%	59.5%	**0.000**
NON CARRIERS OR UNDEFINED CARRIER STATUS (n° 97)								
7 - 14	56	**58.9%**	**57.1%**	55.4%	46.4%	**68.7%**	51.8%	Xx
15 - 27	61	**68.9%**	**65.6%**	60.7%	57.4%	**63.9%**	41%	Xx
28 - 100	**26**	**26.9%**	**19.2%**	**23.1%**	**23.1%**	**15.4%**	**19.2%**	Xx
> 100	**10**	**10%**	**10%**	**20%**	**10%**	**0%**	**0%**	Xx

a) including 4 cattle with questionable carrier status, and negative for all tests in both dpi class

Evidence that virus persistence maintains the level of Ab to NSP detectable for long time - In cattle that recover from infection Ab to NSP decay more rapidly

Diagnostic Sensitivity - RESULTS

VACCINATED CATTLE EXPOSED TO INFECTION
without evidence of infection

Days after exposure	N° cattle	NCPanaf-screening	IZS-Brescia	Ceditest	Svanovir	Chekit	UBI	p value
ALL, NO EVIDENCE OF INFECTION OR UNKNOWN INFECTIOUS STATUS (n° 121)								
7 - 14	91-92	41.3%	48.9%	43.5%	34.8%	42.9%	18.5%	Xx
15 - 27	34	32.4%	26.5%	26.5%	29.4%	26.5%	11.8%	Xx
28 - 100	16	37.5%	50.0%	31.3%	*62.5%*	31.3%	25.0%	Xx
> 100	0	-	-	-	-	-	-	-
CATTLE WITH NO EVIDENCE OF INFECTION (VI neg/PCR neg) (n° 17)								
7 - 14	15	40%	45,7%	20%	53,3%	33,3%	26,7%	Xx
15 - 27	8	25%	12,5%	12.5%	12,5%	12,5%	0%	Xx
28 - 100	15	33,3%	46,7%	26,7%	*66,7%*	26,7%	20%	Xx
> 100	0	-	-	-	-	-	-	-

Finding of seroreactors to NSP; yet lacking any other evidence of infection: NSP serology may help to infere an infection condition that could be missed by any other test system

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Diagnostic Sensitivity Conclusions

Positive Sensitivity covariance: tests results are highly correlated among the six tests

The production of antibody to NSP is correlated to the extent of virus replication

Cattle non protected by vaccination and or carriers are more likely to be detected. *Sensitivity reaches 100% with any test in non vaccinated and infected cattle and approaches 90% with the better tests in vaccinated/carriers*

2 NSP tests show sensitivity performance comparable to the OIE index test (PANAFTOSA)

- **CEDI Diagnostic (commercial kit)**
- **Brescia ELISA (in-house assay)**

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Seroprevalence rates detected in post outbreaks surveys

	PANAFTOSA	BRESCIA	CEDITEST	SVANOVA	CHEKIT	UBI
Israel	**25.8 %**	**25.6 %**	**22.4 %**	**21.3 %**	**20.9 %**	**15.7 %**
Zimbabwe	**67.7 %**	**65.2 %**	**66.2 %**	**50.5 %**	**48.8 %**	**53.5 %**

Origin	N°	Level of agreement		
		Positive in 6 tests	Negative in 6 tests	Discordant in 1 or > test
Israel	**465**	**12.3 %**	**66 %**	**22.7 %**
Zimbabwe	**402**	**37.6 %**	**22.9 %**	**39.5 %**

Analysis of discrepancies-concordance

PANAFTOSA; Ceditest and Brescia ELISAs contributed the higher number of positive-concordant results (higher relative sensitivity and concordance)

NSP tests diagnostic performance in post outbreak surveys - Conclusions

Higher detection rates and concordance shown by three tests

- PANAFTOSA
- Brescia
- Cedi

consistently with results on experimental models

Results from field samples provided estimates of NSP-antibody prevalence in different field situations and another possibility to compare tests

Due to circulation of FMDV SAT1 and SAT2 in Zimbabwe, the analysis enabled us to extend assays validation to these serotypes

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Diagnostic performance of NSP ELISAs in small ruminants and pigs

	NON EXPOSED		EXPOSED		Total
	Non Vacc.	Vaccinated	Non Vacc.	Vaccinated	
SHEEP SERA	422 a)	12	172	100	706
PIG SERA	130 a)	54	15	70	269

SHEEP	NON INFECTED non vacc. & vacc.		EXPERIMENTALLY INFECTED * Non-Vaccinated 25-28 dpi		Vaccinated 28 dpi		6-8 dpi
NSP-ELISAs	pos./total	Spec. %	pos./total	%	pos./total	%	pos./total
NCPanaftosa	9/434	97.92	9/9	100	3/6	50	4/16
IZS-Brescia	2/428	99.53	9/9	100	4/6	66.66	2/16
Ceditest	0/431	100	9/9	100	2/6	66.66	5/16
Chekit	0/431	100	9/9	100	2/6	33.33	1/16

PIGS	NON INFECTED non vacc. & vacc.		EXPERIMENTALLY INFECTED * Non-Vaccinated > 20 dpi		Vaccinated > 20 dpi		≤14 dpi
NSP-ELISAs	pos./total	Spec. %	pos./total	%	pos./total	%	pos./total
Ceditest	0/152	100	12/12	100	10/18	55.5	5/42
IZS-Brescia	1/179	99.44	12/12	100	8/18	44.4	3/42
Chekit	6/181	96.69	12/12	100	3/18	16.6	2/40
UBI	10/184	94.56	12/12	100	4/18	22.2	2/42

* undefined infection/carrier status

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Seroprevalence rates in sheep samples from affected areas

SHEEP	2004 outbreak non-vacc./pos to SP ISRAEL		2001 outbreak non-vacc./pos to SP UNITED KINGDOM		Serosurvey in vaccinated areas TURKEY [b]	
NSP-ELISAs	pos./total	Seroprev. %	pos./total	Seroprev. %	pos./total	Seroprev. %
NCPanaftosa	59/63	**93.65**	85/100	**85**	37/78	**47.43**
IZS-Brescia	59/63	**93.65**	77/100	**77**	33/78	**42.30**
Ceditest	56/63	**88.88**	60/100 [a]	**60**	36/78	**46.15**
Chekit	55/63	**87.30**	40/100	**40**	38/78	**48.72**

a) test carried out in the laboratory of origin
b) possible bias due to pre-selection of samples on the basis of preliminary testing

✓Homogeneous prevalence found by the 4 tests in the flock in Israel: uniform infectious status of animals within the flock
✓Significant inter-assays variation in sheep from UK epidemic : bias?
✓Lower prevalence of antibodies in sheep from Turkey: vaccinated regions?

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Evaluation of serological tests in pigs using field sera from HK-SAR

Dónal Sammin

Introduction

- Insufficient sera from pigs and sheep
- Field collection as alternative to experimental challenge studies
- Endemic area with large numbers of vaccinated pigs and FMD outbreaks regularly reported → HK-SAR

Materials and methods

- LoA with AFCD (HK) and IAH-P
- AFCD to provide specimens from >300 pigs
- IAH-P to perform serological tests

Specimen collection

- Several locations
- Confirmed outbreaks (virus typed)

- > 100 Vaccinated pigs - uninfected
- > 100 “Convalescent” vaccinates
- > 100 “In-contact” vaccinates

Prospective collection

- AFCD vets to investigate all suspect outbreaks of FMD in HK pigs from December 2004
- Eartagging of affected pigs and unaffected herdmates
- Collection of lesion material
- Laboratory confirmation of diagnosis (Ag ELISA)

>= 30 days later: return visit to collect sera and saliva from eartagged pigs + pay “compensation” to herdowner

Laboratory tests

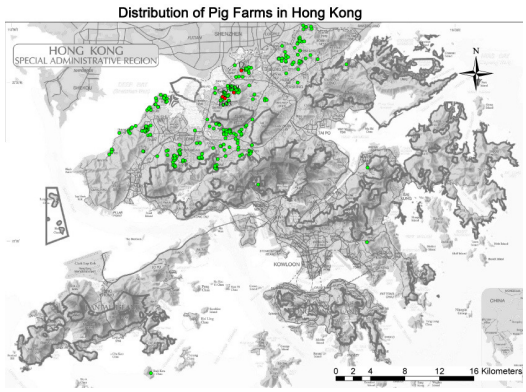
- Virus detection
  AFCD (HK) – Ag ELISA
  IAH-P (UK) – Ag ELISA, RT-PCR, VI
- Serology (IAH-P, UK)
  VNT and SPCE (serotype O)
  NSPE x 3 (cedi, bommeli and UBI tests)

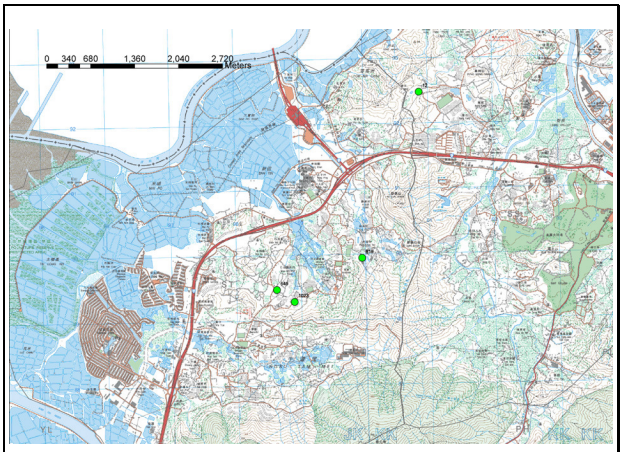
UBI test

- "screening" and "neutralisation" formats
- UBI-screening assay (relatively low SP)
- UBI-S^+ but $Cedi^-$ sera retested by UBI-N

Herds

- 4 herds sampled early March 2005
- n = 405 pigs

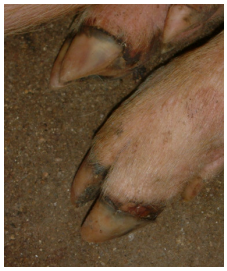




Herd A Control

- 6000 pigs
- 1.3, 1.4 and 2.7 km from B, C and D
- vaccinated 11 and 20 wks
- DOE vaccine (type O)
- no clinical signs of FMD
- 100 fatteners; 3-6 months

Herd B "Infected"



- 2800 pigs
- vaccinated (x1) at 80 days
- DOE vaccine (type O)
- FMD 28/12/04 – 27/02/05
- 80% morbidity
- 5% mortality
- Lesion material x 2 pigs
- 72 pigs @ 30-64 days

Herd C "Infected"

- 4100 pigs
- Same owner as Herd B
- vaccinated (x1) at 80 days
- FMD? 30/12 – 05/01/05
- 95% morbidity? (15% mortality)
- NO Vet. investigation
- NO lesion material collected
- NO hoof lesions
- 82 fattening pigs @ 60 days

Herd D "Infected"

- 1900 pigs
- vaccinated x2; 75 and 110 days
- FMD 16/02 – 24/02/05
- 13% morbidity
- 0.4% mortality
- Lesion material x 1 pig
- 151 pigs @ 30 days:

"Convalescent" (n = 50)
"In-contact" (n = 101)

Virus detection

HERD	ACE (HK)	ACE (IAH-P)	RT-PCR	VI
A				
B (n = 2)	+ (O)	+ (O)	+	+ (O)
C				
D (n = 1)	-	+ (O)	+	-

Serology (% seropositive)

	VNT	SPCE	Cedi	UBI-S	Chekit
A (n = 100)	13	47	0	5	0
B (n = 72)	56	83	49	53	21
C (n = 82)	7	20	0	27	0
D (n = 151)	54	83	27	46	14

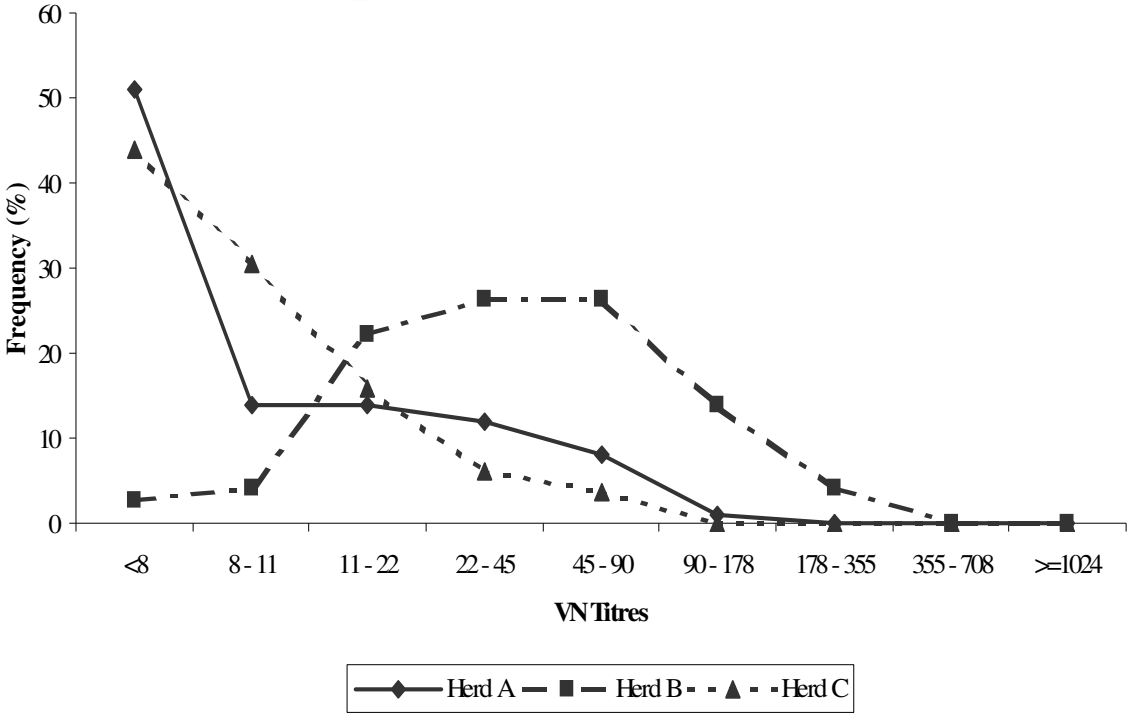
UBI-S^{+}/Cedi^{-} "discrepant" sera (n = 64)

	UBI-S+/Cedi-	UBI-N^{+}
A (n = 100)	5	0
B (n = 72)	8	6
C (n = 82)	22	1
D (n = 151)	29	7

Serology in Herd B (% seropositive)

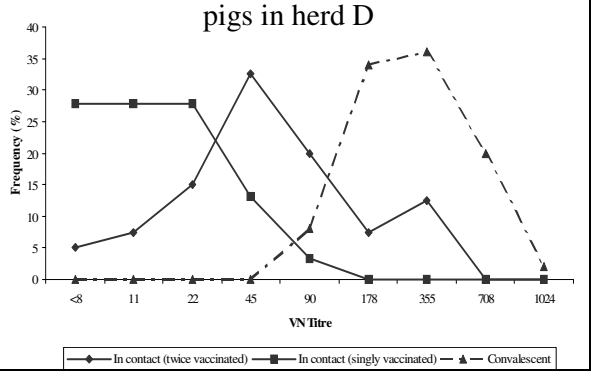
	VNT	SPCE	Cedi	UBI-S	Chekit
Convalescent gilts (8 – 12m) n = 20	85	100	30	30	10
Convalescent fatteners (5-7m) (n = 52)	44	77	56	62	25

VN profiles: A, B & C



Serology in Herd D (% seropositive)					
	VNT	**SPCE**	**Cedi**	**UBI-S**	**Chekit**
In-contact Vaccinated x1 (n = 61)	11	56	0	21	0
In-contact Vaccinated x2 (n = 40)	60	93	0	30	0
Convalescent (n = 50)	100	100	82	88	42

VN profiles: convalescent and in-contact pigs in herd D



Conclusions

- True status of herds A and C uncertain
- Presence of hoof lesions correlates with NSP AB^+
- Relative Sensitivities:
  SPCE > VNT; Cedi = UBI > Chekit
- Specificity of UBI-S assay?
  UBI-N assay ↑ SP but ? ↓ SE
- Efficacy of vaccination regimes?
  Inadequate "protective" AB response in singly-vaccinated pigs

Application of FMDV non-structural protein antibody tests in helping to demonstrate disease-freedom after outbreaks of FMD in cattle

DJ Paton, K De Clercq, M Greiner, A Dekker, E Brocchi, I Bergmann, D Sammin, S Gubbins

Talk overview

- Purpose of testing – demonstrating freedom from infection
- Brescia workshop results
- Methods to optimise overall test specificity and sensitivity
- Factors affecting confidence in testing system
- Examples of what might be possible

Demonstrating freedom from infection

- Purpose is to demonstrate freedom of infection at the herd and population level by testing individuals then extrapolating to herds and populations
- An infected animal can be in one of several states and need to decide which ones to detect
  - Recovered
  - Acutely infected
  - Persistently infected
- Particular interest is use after emergency vaccination
- Unless all animals are tested with a perfect test one cannot give an absolute guarantee of freedom from infection – confidence and design prevalence
- What level of confidence is desired – should relate to risk ?
- Testing is only one part ~ 'substantiation' and not 'demonstration'
- Available guidance from OIE and EU Directive

Brescia Workshop Results (Sp)

Diagnostic specificity in non-vaccinated and vaccinated cattle

Vaccination status	N° cattle	NCPnaftosa screening	IZS-Brescia	Cedtest	Svanovir	Chekit	UBI
FIRST TEST							
Non vaccinated	675	97.3%	97.3%	97.2%	98.7%	98.2%	99.0%
vaccinated	425	96.9%	97.4%	99.5%	98.1%	96.7%	97.9%
All	1100	97.2%	97.4%	98.1%	98.5%	97.6%	98.5%
RETEST OF POSITIVE REACTING SERA							
Non vaccinated	675	98.8%	99.9%	99.0%	99.1%	98.8%	99.4%
vaccinated	425	97.8%	99.5%	99.5%	98.8%	98.8%	98.4%
All	1100	98.1%	99.7%	99.2%	99.0%	98.8%	99.0%

Brescia Workshop Results (Se)

Comparative diagnostic sensitivity in non vaccinated and vaccinated cattle exposed to experimental infection

Days after exposure	N° cattle	NCPnaftosa-screening	IZS-Brescia	Cedtest	Svanovir	Chekit	UBI
NON-VACCINATED CATTLE EXPOSED TO INFECTION (n = 54)							
28 - 100	26	100.0%	100.0%	100.0%	96.2%	92.3%	100.0%
> 100	2	100.0%	100.0%	50.0%	50.0%	50.0%	50.0%
VACCINATED CATTLE EXPOSED TO INFECTION, INCLUDING ONLY CATTLE WITH EVIDENCE OF INFECTION							
28 - 100	92	75.0%	67.4%	68.5%	57.6%	53.3%	60.9%
> 100	47	72.3%	63.8%	74.5%	57.4%	38.3%	46.8%
VACCINATED CATTLE EXPOSED TO INFECTION, KNOWN TO BE PERSISTENTLY INFECTED AT 28 DAYS POST INFECTION OR LATER							
28 - 100	**66** [a]	**93.9%**	**86.4%**	**86.4%**	**71.2%**	**68.2%**	**77.3%**
> 100	37 [b]	89.2%	78.4%	89.2%	70.3%	48.6%	59.5%

a) : including 4 cattle with questionable carrier status, and negative for all tests at all post infection time points, except one sample in class 28-100 positive in Svanovir test.

b) : including 15 cattle with no evidence of infection.

Methods to optimise test specificity (and sensitivity)

- Retesting with the same test to counter failures of reproducibility
- Parallel testing to increase sensitivity
- Serial testing – i.e. confirmatory testing with another NSP
- Conditional dependence of different tests
  - Sensitivity covariance
  - Specificity covariance
- Alter or vary test cut-offs based on ROC and likelihood ratio analysis
- Confirmatory testing with other tests
  - Use of EITB
  - Use of salivary IgA test
- Resampling and retesting
  - Same or other animals and herds
  - Probang testing for direct virus detection
- Cluster analysis – animals and herds

Factors affecting confidence

- Test system sensitivity and specificity
- Size of herd or epidemiological unit
- Sample size
- Prevalence or absolute number of infected animals to be detected in a herd
- Prevalence of infected herds
- Population characteristics
- Survey design and representation/coverage

Detection of carriers in vaccinated cattle

Specificity and sensitivity for detection of individual carrier cattle when sampling at 28-100 days post exposure using different combinations for sequential testing of positive samples

Test system	**Specificity* (%)**	**Sensitivity** (%)**
Cedi screen	98.1	86.4
Cedi screen plus Cedi retest	99.2	85.1
Cedi screen plus Cedi retest plus Panaftosa retest	99.98	79.8
Cedi screen plus Cedi retest plus Svanova retest	99.98	71.2
Cedi screen plus Cedi retest plus probang/RT-PCR test	100	69.8

Detection of carriers in unvaccinated cattle (a) assuming 100% Se of tests

Specificity and sensitivity for detection of individual carrier cattle when sampling at 28-100 days post exposure using different combinations for sequential testing of positive samples

Test system	**Specificity* (%)**	**Sensitivity** (%)**
Cedi screen	98.1	100
Cedi screen plus Cedi retest	99.2	99
Cedi screen plus Cedi retest plus SPCE retest	99.996	99
Cedi screen plus Cedi retest plus SPCE retest plus probang/RT-PCR test	100	81

Detection of carriers in unvaccinated cattle (b) assuming 90% Se of tests

Specificity and sensitivity for detection of individual carrier cattle when sampling at 28-100 days post exposure using different combinations for sequential testing of positive samples

Test system	**Specificity* (%)**	**Sensitivity** (%)**
Cedi screen	98.1	90
Cedi screen plus Cedi retest	99.2	89
Cedi screen plus SPCE retest	99.99	81
Cedi screen plus SPCE retest plus probang/RT-PCR test	100	66

Sample sizes, percentages of tested herds likely to give one or more false positive results and reactor cut-points when using different test combinations to detect 5% infection at 95% confidence in primovaccinated cattle

Herd size	Test system											
	Cedi/Cedi retest 99.2% Sp & 85% Se			Cedi/Cedi retest/ Panaftosa confirmation 99.98% Sp & 79.8% Se			Cedi/Cedi retest/Svanova confirmation 99.98% Sp & 71.2% Se			Cedi/Cedi retest/Probang RT-PCR confirmation 100% Sp & 69.7% Se		
	Sample size	% false positive herds	Cut point	Sample size	% false positive herds	Cut point	Sample size	% false positive herds	Cut point	Sample size	% false positive herds	Cut point
10	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	30	0.6	0	-	-	-	-	-	-
60	-	-	-	48	1.0	0	53	1.1	0	55	0	0
100	90	52	2	56	1.1	0	63	1.3	0	65	0	0
200	128	64	3	64	1.3	0	72	1.4	0	74	0	0
400	140	68	3	69	1.4	0	77	1.5	0	79	0	0

Minimum herd sizes for each test system are 91, 30, 51 and 51 respectively
Sample size is number of cattle from which samples must be collected to detect 5% prevalence with 95% confidence.
% false positive herds is proportion of uninfected herds expected to give at least one positive seroreactor.
Cut point is maximum number of positive seroreactors indicative of <5% prevalence of infection.

Sample sizes, percentages of tested herds likely to give one or more false positive results and reactor cut-points when using different test combinations to detect 5% infection at 95% confidence in non-vaccinated cattle

a) Assuming 100% Se of Cedi and SPCE and 99% Se of Cedi/Cedi retest

	Test System		
	Cedi/Cedi retest/SPC ELISA confirmation 100% Sp & 99% Se		
Herd size	**Sample size**	**% false positive herds**	**Cut point**
10	-	-	-
30	24	0	0
60	38	0	0
100	45	0	0
200	52	0	0
400	55	0	0

Minimum herd size is 11

b) Assuming 90% Se of Cedi and SPCE

	Test System					
	Cedi/SPC ELISA retest 99.99% Sp & 81% Se			Cedi/SPC ELISA retest/Probang RT-PCR confirmation 100% Sp & 66% Se		
Herd size	**Sample size**	**% false positive herds**	**Cut point**	**Sample size**	**% false positive herds**	**Cut point**
10	-	-	-	-	-	-
30	29	0.3	0	-	-	-
60	47	0.5	0	58	0	0
100	55	0.5	0	68	0	0
200	63	0.6	0	78	0	0
400	68	0.7	0	84	0	0

Minimum herd sizes are 30 and 53 respectively

Sample size is number of cattle from which samples must be collected to detect 5% prevalence with 95% confidence.
% false positive herds is proportion of uninfected herds expected to give at least one positive seroreactor.
Cut point is maximum number of positive seroreactors indicative of <5% prevalence of infection.

Conclusions

- Combinations of available NSP tests used in series give high Sp, but Se is limiting and may cause problems to reach desired confidence of detecting low levels of carriers in small herds
- Resampling and retesting - OP testing with RT-PCR appears to offer few benefits (and serious consequences), but value of repeat sampling for serology needs to be quantified
- Consequences of different findings need to be elaborated
- Still uncertainty over design appropriate design prevalences and over desired level of confidence (which should relate to risk)
- Testing is only one part of risk mitigation process and other specific measures could be considered, e.g. special precautions for entire bulls
- Some more testing and analysis is needed for sheep and pigs
- Various options are available to deal with the small-herd problem

Acknowledgements

- DG Research (*FMD ImproCon*)
- DG Sanco
- EUFMD

Appendix 15

Application of optimal sampling strategies for substantiating disease freedom

Matthias Greiner

International EpiLab, Danish Institute for Food and Veterinary Research, Mørkhøj

Bygade 19, DK-2860 Søborg, Denmark

David Paton

Institute for Animal Health, Ash Road, Pirbright, Surrey, GU24 ONF, UK

Abstract

We describe a method for optimising the application of a diagnostic test for surveillance purposes. The objective is to establish a procedure for maximising the herd level sensitivity by choosing a sample size (within herd) and a cutpoint (number of test positive animals required to classify a herd as positive) for each herd size under the constraint that the herd specificity is greater than a specified minimum value. The second objective is to use such herd size-specific sensitivities to assess the performance of a surveillance system. This includes sample size (among herds) calculations and the estimation of the confidence, i.e. the probability to detect at least one infected animal in a population, given a specified level of prevalence. The methods are illustrated using the example of post-vaccination surveillance for foot-and-mouth disease.

1. Introduction

The methodology for substantiating freedom from disease can be derived from the concept of diagnostic sensitivity. For example, Martin et al. (2005) define the term "Surveillance component sensitivity" as the probability that a given surveillance component detects at least one truly infected herd, given that infected herds in the population and infected animals in infected herds occur at levels specified by the among-herd and within-herd "design prevalence", respectively. The surveillance sensitivity is sometimes referred to as "confidence" or "power" and depends on the sensitivity and specificity of the diagnostic procedure used, the number of reactors required to classify the herd as infected (herd cutpoint) and the sampling scheme for herds and animals within herds. The statistical methods used in the context of disease freedom are described in detail by Cameron and Baldock (1998a), Cameron and Baldock (1998b), Cannon (2001) and Cannon and Roe (1982).

Paton et al. (2004) have recently described the rationale for surveillance for foot-and-mouth disease (FMD) to regain the status of a country or region as free without vaccination after an outbreak, which has been controlled by measures including vaccination. It is an essential requirement for surveillance programmes in this context that the presence of persistently infected (carrier) cattle in the population can be detected with high (say 95%) level of confidence. The design prevalence in this case refers to the presence of such carrier animals in infected, vaccinated or unvaccinated herds. While the level of the confidence is set by

international requirements, a certain specificity of the surveillance programme must be maintained for economic reasons. Brocchi et al. (2005) have provided estimates of the diagnostic sensitivity and specificity of different non-structural protein (NSP) ELISAs that are candidate tests for the use in post-outbreak FMD surveillance. The implementation of these tests in surveillance programmes can be as single or multiple tests as outlined by Paton et al. (2004). Important parameters for the performance of a surveillance programme are the herd-level sensitivity and specificity. These parameters depend on the diagnostic test performance on the animal-level, the herd cutpoint, the herd size and the herd sample size (see Jordan and McEwen (1998)).

We describe a method to identify an optimal combination of a sample size and herd cutpoint for given herd sizes and test performance characteristics, which would maximise the herd sensitivity while a specified minimum herd specificity is maintained. An application of such a herd-specific sampling and interpretation scheme is investigated and discussed using a simulated post-outbreak FMD surveillance as case study.

2. Methods

2.1 Notation and scenarios

The case study considers surveillance for the stratum of vaccinated herds. The scenarios considered in this study are defined by the the animal-level sensitivity *(Se)* and specificity *(Sp)* of the diagnostic procedure. In scenario 1 we consider the use of the Cedi test for screening and retesting of initial positives. For this procedure we assume $Se_1 = 0.851$ for detection carrier animals and $Sp_1 = 0.981$. In scenario 2 we use the Cedi test for screening and retesting of initial positives and additional retesting with the Svanova test, which has $Se_2 = 0.712$ and $Sp_2 = 0.99$. Brocchi et al. (2005) have estimated the sensitivity and specificity covariance of the Cedi and Svanova test for vaccinated cattle as $\gamma Se = 0.164$ and $\gamma_{Sp} = 0$, respectively. The estimated sensitivity covariance exceeds the maximum possible value $\max(\gamma_{Se}) = \min [Se_1 (1-Se_2), Se_2(1 - Se_1)] = 0.016$ (Gardner et al. (2000)). The sequential testing (with confirmation of initial positives) implies the diagnostic parameters for scenario 2, $Se = Se_1Se_2 + \gamma_{Se} = 0.7120$ and $Sp = 1 - (1 - Sp_1)(1 - Sp_2) - \gamma_{Se} = 0.9998$. For both scenarios, we assume a within herd design prevalence of $p_A = 0.05$ and a between herd design prevalence of $p_H = 0.02$. The diagnostic status of interest is the persistent infection (carrier status) and the population is assumed to be vaccinated. We use two levels of the minimum required herd specificity (min Sp_H), 95% and 99%. Other input parameters include the herd cutpoint c, the herd size N and the herd sample size n.

2.2 Optimisation of the herd testing

The herd sensitivity (Se_H) and specificity (Sp_H) is modeled using the hypergeometric distribution as described by Jordan and McEwen (1998) with the following modification. The number of truly infected animals in the herd, given the herd is infected, is $\max(1, Np_A)$, i.e. infected herds have at least one infected animal regardless of the herd size. The optimisation of Se_H for each herd size was achieved by searching for a combination (n, c), which leads to maximum values for Se_H, while Sp_H is greater than the specified minimum level minSp_H. When multiple combinations fulfil these criteria, the one with the smallest sample size was chosen. The outcome of the procedure is a list of values for N, n, c, Se_H and Sp_H, where Se_H is the optimised herd sensitivity and (n, c) is the optimal herd testing scheme for herd size N.

2.3 Estimation of the surveillance performance

Due to the fact that Se_H is specific for each herd size, the population sensitivity of the surveillance system (Se_S) and the number od herds to be tested (h) to achieve specified values for Se_S can only be found by simulation. Briefly, in is order to estimate Se_S as a function of h, the number of infected herds out of h was simulated using Poisson random number with parameter (hp_H). From the distribution of observed herd sizes, a number of h herd sizes was chosen with sampling probability as given by the empirical relative frequency distribution of herd sizes. The optimised Se_H was established for those selected, infected herds as described above. The Se_S estimate for one iteration is just 1 minus the probability that none of the h herds is infected and detected. The mean of this value over 1000 iterations is the simulation estimate of Se_S. The number of herds to be tested (h) to achieve a specified value of the surveillance sensitivity (Se_S) is found by increasing h stepwise until 950 out of 1000 simulated surveillance procedures resulted in a "success". A successful iteration is defined as one in which the probability to select and detect at least one infected herd out of h randomly chosen herds exceeded the pre-specified value for Se_S. The required sample size h is the smallest sample size for which 950/1000 successes were observed. The expected number of animals to be tested in the surveillance is $h\Sigma_k\, pn$, where k denotes the category of a herd size and p and n denote the relative frequency and the optimised sample size of the kth category, respectively. The simulation process is repeated 100 times and the 5th, 50th and 95th percentile of all outcomes are reported as final results.

2.4 Implementation

All procedures were implemented in the R programming environment (R Development Core Team, 2003) in form of the programme "herdplus", which contains the modules "input" (for parameter input), "herdtest" (returns Se_H and Sp_H), "mat" (displays matrix of Sp_H and Se_H for a range of n and i), "opt" (optimises n and c for each herd size N), "appl" (applies optimised scheme in a simulated surveillance). The programme herdplus and the programme environment R can be obtained free of charge from the first author and from http://www.R-project.org, respectively.

2.5 Data

For the case study, the number of cattle registered in 2004 on a total of 19030 holdings in Jytland, Denmark (extract from central husbandry register) were used to tabulate the relative frequency of all observed herd sizes (not shown).

The minimum, 5th, 50th, 95th percentile, mean and maximum herd size is 1, 2, 27, 279, 76.2, 1661, respectively.

3 Results

3.1 Optimised herd testing

For illustration, optimised herd testing schemes are given for a selection of herd sizes using the parameter sets of scenario 1 and 2 (Tab. 1). With the herd specificity constraint set to 95% and for a herd size of 27, which is the median herd size in the study data, a maximum herd sensitivity of 63.6% can be reached when all 27 animals are tested and herds with $c = 2$ or more reactors are scored positive. Due to the better specificity in scenario 2, a cutpoint of $c =$

1 can be used for the same herd size, which leads to improved herd sensitivity although the animal level sensitivity is lower than in scenario1. This effect applies also to larger herd sizes, where the higher specificity leads to smaller sample sizes. For example, optimal sample sizes for a herd size of 279 are 224 and 166 for scenarios 1 and 2, respectively. The effect of setting the minimum herd specificity to 99% is either a reduced sample (leading to reduced herd sensitivity) or increased cutpoint or a combination of both.

A numerical example of the target function (Se_H) over the optimisation range (n, c) is given for the example of a herd size of 76 (Tab. 2). The range of c = 1, 2 and n = 70, . . . , 76, reaches perfect herd sensitivity but is rejected due to failure of reaching the minimum specified herd specificity. The optimal testing scheme as reported in Tab. 1 can be confirmed. For further use, the optimal combination (n, c) is established for all herd sizes found in the study data. For scenario 1 with a 95% herd specificity constraint, a weighted mean herd sensitivity of 0.7046 is reached. The herd sample size and diagnostic parameters are plotted against herd sizes in Fig. ??.

3.2 Simulated surveillance

A number of 275 herds (95% simulation CI: 265, 283) has to tested in scenario 1 with minimum herd specificity set at 95% to reach a population surveillance sensitivity of 95%. The number of animals tested is 14908 (CI: 13211, 17081). Results for the other scenarios are pending. Furthermore, the effect of restricting the surveillance to herds that are not small and the effect of using herd-size category specific rather than herd size specific testing schemes could be investigated. The final version may also look into the effect of using stochastic rather than fixed values for *Se* and *Sp*.

4. Discussion

Standard methods for the design and analysis of surveillance systems use constant herd sample sizes for the entire population or for categories of herd sizes. This neglects the possibility to obtain improved diagnostic performance in the herd classification by choosing optimal herd sample sizes and herd cutpoints. Reasons for not making full use of the potential diagnostic capacity of a herd testing scheme may be of practical nature. Firstly, it may be difficult to work with herd specific sample sizes and interpretation rules. In the future, automated processes of sampling, testing and interpretation may greatly reduce these complications. Secondly, suitable software and clear objectives for the optimisation have been lacking. We have chosen as optimality criterion a maximum sensitivity, under the constraint of a specified minimum herd specificity. We chose the herd sensitivity as outcome of the optimisation rather than as fixed constraint. This is justified in a population approach to substantiating disease freedom, where individual infected herds may be misclassified as "negative", as long as, with high probability, one or more infected herds are correctly classified in the whole population. It is obvious that this algorithm is especially tailored for the purpose of substantiating absence of infection from a population. Other purposes, such as for example the control of outbreak or eradication of endemic diseases would require a different approach. In such cases, alternative optimality criteria for herd classification could be derived from weighted summary indices of herd sensitivity and specificity, where the weights reflect some economic utilities.

We have illustrated the impact of optimised herd testing schemes using selected herd sizes and showed that diagnostic systems with high animal-level specificity, such as for example sequential testing strategies as in scenario 2, may be advantageous in terms of herd-level sensitivity. Our optimisation algorithm provides insight in the background of this effect. The high specificity in the classification of individual animals allows a lower cutpoint be selected, which in turn increases the herd sensitivity. The use of a lower cutpoint, on the other hand,

reduces the required sample size, without compromising herd sensitivity. This is in contrast to the common notion that herd sensitivity increases with lower animal-level specificity due to false positive results that eventually contribute to the correct identification of an infected herd (Christensen andGardner (2000)). Our optimised testing schemes seem to over-compensate for this effect.

The optimised sampling scheme was applied in a virtual surveillance. The required sample size for reaching the pre-specified confidence level of classifying

Further discussion points:

- In the case study, the assumption was made that all herds share the same characteristics. In reality, this may not be the case. For example, small herds may not be subjected to vaccination and may be tested using different protocols. This would constitute a separate surveillance component, which would be analysed seperately but with the same tools as described.
- The herd testing scheme can be controlled with further constraints. For example, herdplus allows maximum sample sizes being set. This would result in a limitation of the sample size at the cost of a reduced herd sensitivity. The option is not used in the case study.
- The stochastic simulation used in this study is meant to mimic an actual surveillance process. Therefore, we modeled discrete probabilistic statuses and events of individual herds and animals rather than assigning probabilities to these objects. For example, an individual animal is either infected (status assigned with probability of within herd prevalence) or non-infected. Only animals assigned as infected would contribute to the probability of correct identification of a herd if perfectly specific tests were used.
- Effect of using stochastic values for Sc and Sp should be discussed.

5. Conclusion

The performance of a surveillance system can be improved in several ways. Given a diagnostic test with certain values of diagnostic sensitivity and specificity, one can, for example, choose a herd testing scheme that optimises the herd sensitivity while keeping the herd specificity above a specified minimum level. This involves sample sizes and cutpoints (number of positive results required to classify a herd as positive) that are specific to any given herd size. The performance of a surveillance system based on an optimised herd testing scheme can be characterised in terms of the surveillance sensitivity, which is the probability that the surveillance finds at least one infected animal, given the population is infected at the level of a specified design prevalence. We have described a software tool for this kind of optimisation and applied it in a case study on post-outbreak surveillance for FMD in Danish cattle herds.

6. Acknowledgements

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Table 1

Sample sizes n and herd cutpoints c for herds with size N, which result in maximum herd sensitivity Se_H while the herd specificity 8 is greater 95%[a]

Scenario 1 (Se = 0.8510, Sp = 0.9810)					Scenario 2 (Se = 0.7120, Sp = 0.9998)			
N[b]	n	c	Se_H	Sp_H	n	c	Se_H	Sp_H
2	2	1	0.8700	0.9620	2	1	0.7122	0.9996
27	27	2	0.6362	1.0000	27	1	0.9663	0.9946
76	75	3	1.0000	1.0000	75	1	1.0000	0.9850
279	224	7	1.0000	1.0000	173	1	1.0000	0.9654

[a] Results (N, c, Se_H, Sp_H) for combinations that failed the a 99% herd specificity requirement were (2, 1, 1, 0.435, 0.981) for scenario 1 and (76, 76, 2, 1, 1) and (279, 203, 2, 1, 1) for scenario 2.

[b] Herd sizes represent 5th and 50th percentile, mean, 95th percentile.

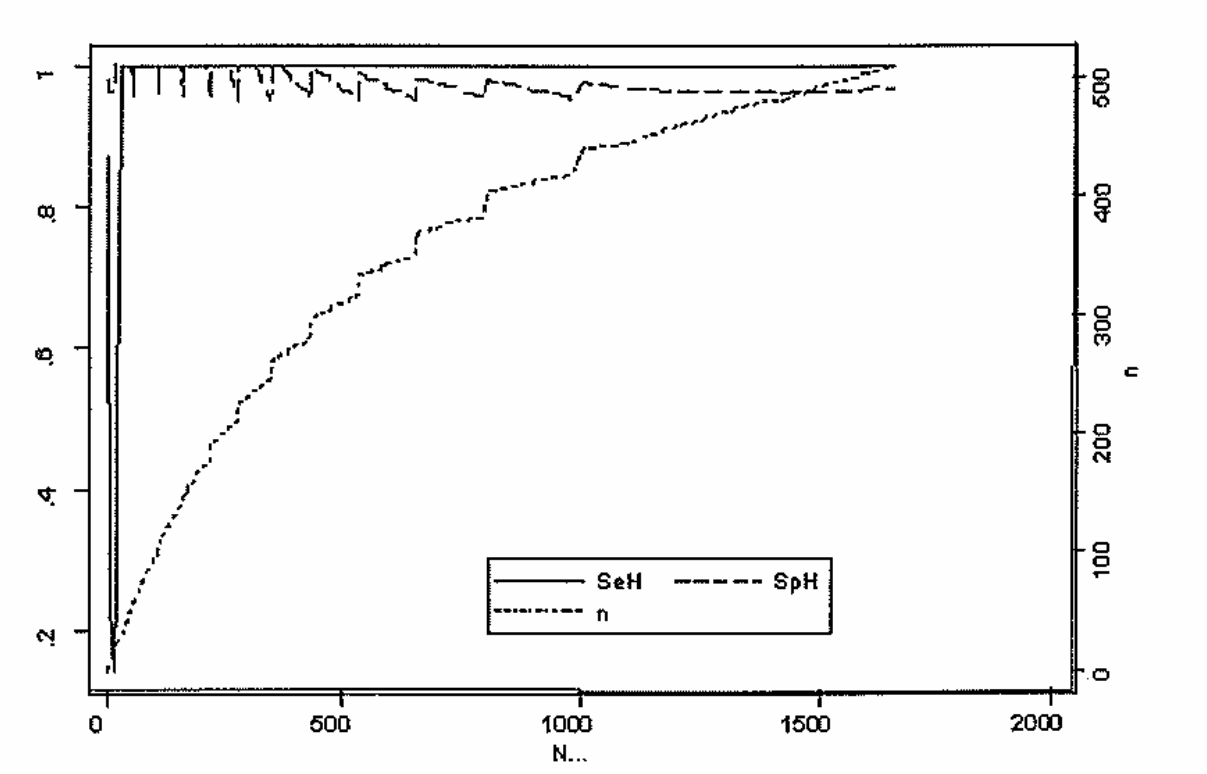


Fig. 1. Optimised herd sensitivity, specificity and herd sample size as function of the herd size for a hypothetical post-outbreak sur veillance for FMD in Denmark.

Table 2

Herd sensitivity and specificity for different sample sizes (n) and cut-points (c) for a herd of size 76[a]

	Herd sensitivity Se_H			Herd specificity Sp_H		
n	*c = 1*	*c = 2*	*c = 3*	*c = 1*	*c = 2*	*c = 3*
70	1.000*	1.000*	0.987	0.046*	0.624*	1.000
71	1.000*	1.000*	0.991	0.038*	0.613*	1.000
72	1.000*	1.000*	0.995	0.030*	0.602*	1.000
73	1.000*	1.000*	0.998	0.022*	0.591*	1.000
74	1.000*	1.000*	0.999	0.015*	0.579*	1.000
75	1.000*	1.000*	1.000	0.007*	0.568*	1.000
76	1.000*	1.000*	1.000	0.000*	0.556*	1.000

[a] Underlying assumptions of scenario 1; see text.

* Combination rejected because *SpH* <0.95

Use of likelihood ratios in analysis of NSP test results

Dónal Sammin

What are likelihood ratios?

- Interpretation of diagnostic test using a single cut-off value →
- ALL are either "Positive" or "Negative" regardless of magnitude of test result
- SE and SP as measures of test performance
- LRs can be used for: (i) test interpretation and/or (ii) measuring test performance

What are likelihood ratios?

		"FMDV-INFECTED"	
		+	-
TEST	+	a	b
	-	c	d

SE = a/(a+c)
SP = d/(b+d)

What are likelihood ratios?

		"FMDV-INFECTED"	
		+	-
CEDI	+	155	1
	-	113	422

268 "infected" vaccinates
423 uninfected vaccinates

SE = 155/268 = 0.578
SP = 422/423 = 0.997

What are likelihood ratios?

- **LR+** = the likelihood that an infected animal will give a positive test result
  = SE/(1-SP)
- **LR-** = the likelihood that an infected animal will give a negative test result
  =(1-SE)/SP

What are likelihood ratios?

		"FMDV-INFECTED"	
		+	-
TEST	+	a	b
	-	c	d

LR+ = [a/(a+c)]/[b/(b+d)]
LR- = [c/(a+c)]/[d/(b+d)]

What are likelihood ratios?

		"FMDV-INFECTED"	
		+	-
CEDI	+	155	1
	-	113	422

268 "infected" vaccinates
423 uninfected vaccinates

LR+ = [155/268]/[1/423]
= 0.578/0.003 = 245
LR- = [113/268]/[422/423]
= 0.422/0.997 = 0.4

Multiple-level likelihood ratios

	"FMDV-INFECTED"	
	+	-
1	a	b
2	c	d
3	e	f
4	g	h
5	i	j

(a + c + e + g + i) = x
(b + d + f + h + j) = y

LR1 = [a/x]/[b/y]
LR2 = [c/x]/[d/y]
LR3 = [e/x]/[f/y]
LR4 = [g/x]/[h/y]
LR5 = [i/x]/[j/y]

Multiple-level likelihood ratios

Cedi PI	"FMDV-INFECTED"	
	+	-
<20	58	301
20-40	36	109
40-60	49	12
>60	125	1

268 "infected" vaccinates
423 uninfected vaccinates

= [58/268]/[301/423]	= 0.3
= [36/268]/[109/423]	= 0.5
= [49/268]/[12/423]	= 6.5
= [125/268]/[1/423]	= 197

Multiple-level likelihood ratios

Cedi PI	"FMDV-INFECTED"	
	+	-
<20	58	301
20-40	36	109
40-60	49	12
>60	125	1

+ 268 "infected" vaccinates
- 423 uninfected vaccinates

= 0.3 (0.2 – 0.4)	
= 0.5 (0.4 – 0.7)	
= 6.5 (3.5 – 11.9)	
= 197 (28 – 1403)	

Use of LRs in clinical decision making

- **Pretest Odds x LR = Posttest Odds**

Odds = Prob./(1-Prob.); Prob. = Odds/(Odds +1)

LR>1 increases the Odds
LR<1 decreases the Odds

LR >10 or <0.1 really changes the odds!!

Use of LRs in clinical decision making

- Need to define "pretest Odds"
- Can combine LRs for different "tests"
- Need to define "Threshold" posttest odds for action

"Testing" threshold = more testing required to rule-in or rule-out the diagnosis

"Treatment" threshold = treat the patient!

Pretest Odds

Increased odds of FMD infection

- Presence of clinical signs
- Proximity to a known "infected" herd

(e.g. odds in PZ > odds in SZ >odds outside)

- Evidence of contact with an "infected" herd

BUT need to be EXPLICIT! → numerical value

Combination of likelihood ratios

Likelihood of FMD infection increases with:

- Magnitude of test result [LR1]
- Number of positive animals [LR2]

Are the "tests" independent or correlated?

If independent:

pretest odds x LR1 x LR2 →posttest odds

or pretest odds x combined LR →posttest odds

LR based on the number of positive animals in a "sample"/herd

Probability of a True+ve (Infected herds)

↑ as SE↑ and Prev ↑

Probability of a False+ve (Infected and uninfected herds)

↑ as SP↓ and the number tested ↑

LR based on the number of positive animals in a "sample"/herd

	"FMDV-INFECTED"	
	+	-
P (0+)	x	y
P (1+)		
P (2+)		
P (3+)		
P (4+)		

LR = x/y

Combined LR for sample of size "n" tested with the cedi-test

PI	1 +ve	2 +ve	3 +ve	4 +ve
≤20				
20-40				
40-60				
>60				

Posttest Odds

"Testing" threshold = x

"Treatment" threshold = y

If posttest odds:

<x	herd is OK (NO more testing!)
>x but <y	herd requires follow-up (more testing!)
>y	herd is slaughtered ± contact-tracing

Example of the use of an LR approach to clinical decision-making

- Patient: 40-year-old woman
- Presentation: chest pain
- Clinical examination:

Atypical clinical signs → Prob (PI) = 0.01

Pretest odds = 1:100

Clinical signs typical of angina → Prob (MI) = 0.5

Pretest odds = 1:1 → further investigation

Further investigative "tests"

"TEST"	Type of data	Categories
Measure serum CK	Continuous	4-5 intervals → LR1
ECG	Categorical	3-4 levels → LR2
Exercise intolerance	Categorical	3-4 levels → LR3
Angiography	Categorical	3-4 levels → LR4

Serum CK levels

Interval	LR	95% CI
1-120	0.69	0.49 - 0.90
121-240	0.42	0.20 - 0.85
241-360	4.13	1.87 - 8.70
361-480	7.08	2.81 - 17.17
> 480	9.10	4.15 – 19.30

Decision to rule-in or rule-out a diagnosis of MI

IF sequential testing and tests are independent:

Pretest odds x LR1 → posttest odds 1

x LR2 → posttest odds 2

x LR3 → posttest odds 3

x LR4 → posttest odds 4

After each "test" make a decision to:

(1) rule-out diagnosis of MI;

(2) conduct further testing for MI or

(3) start treatment for MI

Use of LRs to compare tests

3 x NSPE (cedi, bommeli & panaftosa)

Brescia workshop data for cattle

268 "infected" vaccinates

423 uninfected vaccinates

LR+ and LR- calculated at cut-off value

Use of LRs to compare tests

TEST	Cut-off	LR+	LR-
Cedi	>=50 PI	245 (34 – 1737)	0.42 (0.37 – 0.45)
Bommeli	>=30 PP	31 (15 – 66)	0.49 (0.43 – 0.56)
Panaftosa	>=20 PP	30 (14 – 64)	0.5 (0.45 – 0.57)

Conclusions

- LRs provide a useful means of combining results from two or more "tests"
- BUT will only be useful for decision-making purposes if "pretest odds" and "threshold" values are quantified
- Develop combined LR for magnitude of test result and number of seropositive animals in the "sample" → "field test" with real data

Appendix 17

Monitoring after FMD emergency vaccination

A. Dekker, CIDC-Lelystad

Introduction

In the Netherlands it has been decided that foot-and-mouth disease (FMD) emergency vaccination shall have a prominent place in the possible control measures that will be applied after an FMD outbreak. Although tests that can differentiate between infected and vaccinated animals (DIVA tests) have been available for several years, no international accepted data on sensitivity and specificity were available. Comparison of various DIVA tests for FMD is part of the European research project "ImproCon" which is financed partly by the European Union. Together with support of the EU commission and the European Commission for control of foot-and-mouth disease an international workshop has been arranged where sera from various countries were brought together and were tested with all available commercial DIVA tests for FMD. The results of this workshop enable us the design a monitoring programme that can be used in the aftermath of an FMD outbreak.

In article 56 of the European directive 2003/85 monitoring after an FMD outbreak using emergency vaccination is described. Together with clinical inspection of all vaccinated herds, a serological monitoring has to be performed. In article 56 two possibilities are described. First sampling all vaccinated herds, in conformity with § 2.2 of Annex III, for 5% prevalence with 95% confidence. A second sampling has been described in which all vaccinated animals and their offspring have to be sampled. In this report we consider the first method acceptable. However, if risk analysis shows that the chance of new outbreaks is not acceptable, the second method should be considered.

In this report we try to determine the consequences of using emergency vaccination on the number of samples that have to be tested in the aftermath of the epidemic.

Materials en methods

Number of vaccinated premises

In the analysis we have considered two situations; firstly the numbers of infected premises as were found in the 2001 FMD outbreak in the Netherlands. For this analysis data were obtained from the national inspection service for livestock and meat (VWA-RVV), containing information on the farms located within a 2 km radius of the infected herds found in 2001. From this data set we removed the farms that occurred again in a 2 km radius of a later outbreak. From this data set we could calculate the number farms and animals that had to be vaccinated during the outbreak, the average number of samples that should be collected in the first screening and how many false positive results will be found resulting in retesting of the farm.
Secondly we used data from the report "Scenario-onderzoek effectiviteit vaccinatie en impact op afzet producten" of the "Iinstitute for Risk Management in Agriculture" (IRMA) (September 2004) to define a number of farms to be tested. We used the 95% upper limit of the number of vaccinated herds in a simulated outbreak in the most densely populated livestock area in the Netherlands (Midden-Nederland) as a scenario with a large outbreak.

Sensitivity en specificity

The results of various cattle sera tested at the FMD DIVA test workshop in Brescia were used. We used the data of 1100 non-infected cattle, 55 exposed and non-vaccinated cattle and 255 exposed and vaccinated cattle. If sera from the same animal at several timepoints after infection were present we used one randomly selected serum from the series selected after exposure. In the analysis of the various DIVA tests for FMD only small differences were found in the sensitivity and specificity of the various commercially available tests. The test produced in the Netherlands (CEDI diagnostics) was one of the best performers. In the calculation we used the data obtained with this test.

Number of samples per farm

For each farm the number of samples necessary to detect 5% prevalence with 95% confidence was calculated with the following formula (Martin et al. 1992).

$$n \geq \left(1-(1-C)^{\frac{1}{Se \times Pr \times N}}\right) \times \left(N - \frac{Pr \times N - 1}{2}\right)$$

Where n is the number of samples needed, C is the confidence level, Se is the sensitivity of the test, Pr is the prevalence which should be detected and N is the total number of FMD susceptible animals on the farm.

Simulation of false positive samples

Based on the specificity of the test the distribution of the number of farms with false positive results was determined using Monte Carlo simulation. For each farm the herd-specificity, i.e. chance of getting only negative results on that farm, was calculated by taking the test-specificity to the power n (number of samples). If a random number was smaller than this chance the farm would not produce a false positive result, if the random number was bigger than this chance the farm would produce false positive results and should be retested.

Results

Number of vaccinated premises

A total of 1099 unique farms were present in the 2 km zones surrounding the Dutch 2001 FMD outbreaks. From 127 of these farms information on the number of animals was missing. In the analysis we assumed no animals were present on these locations. So a total of 972 farms would have been vaccinated if the new strategy had been implemented in 2001. The date and the number of farms that would have been vaccinated is shown in figure 1.

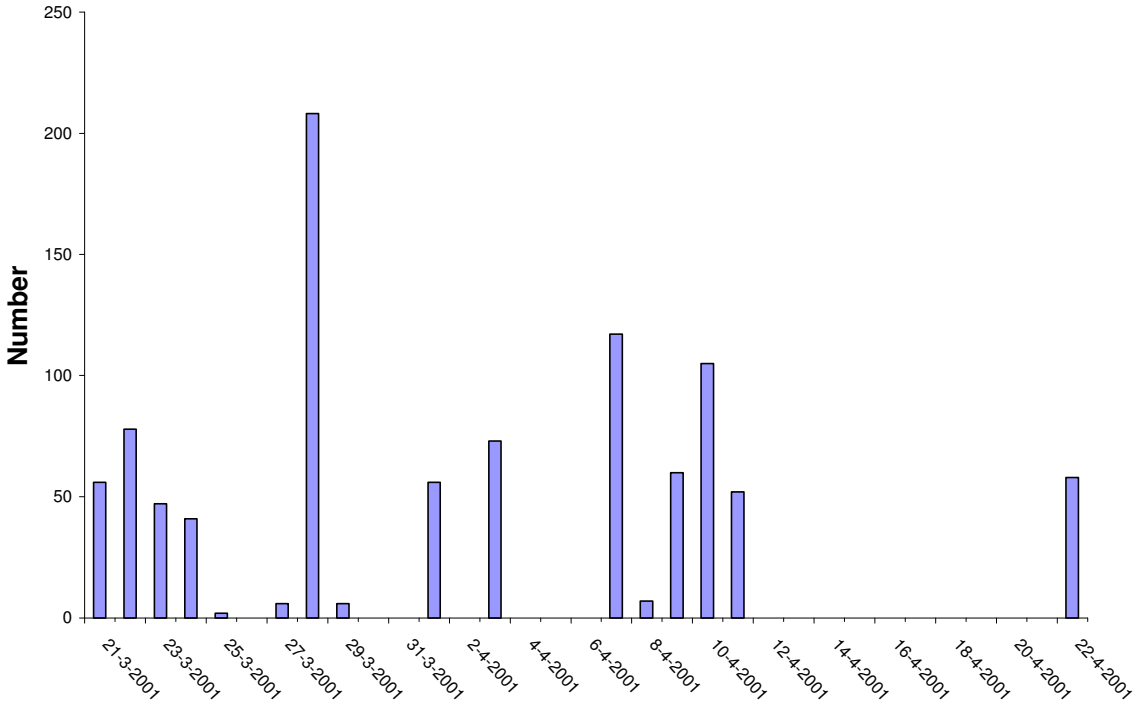
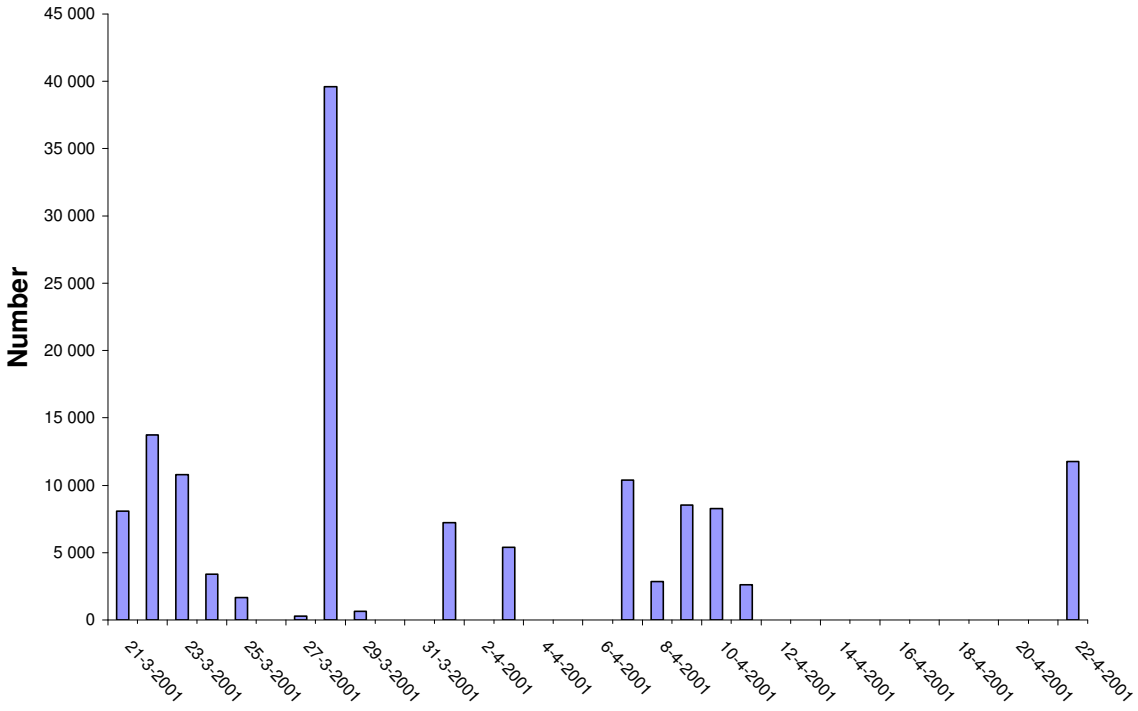


Figure 1: Number of farms where vaccination would have been carried out related to the date of confirmation of the outbreak within the 2 km zone.

A total of 135 197 animals would have been vaccinated. Figure 2 shows the date and number of animals vaccinated.

Figure 2: Number of animals that would have been vaccinated related to the date of



confirmation of the outbreak within the 2 km zone.

In the report "Scenario-onderzoek effectiviteit vaccinatie en impact op afzet producten" the 95% upper limit of the number of vaccinated herds in a simulated outbreak in the densest part of the Netherlands (Midden-Nederland) was 1 210 with a total of 284 132 animals.

Sensitivity en specificity

During the workshop inconsistent results were found, therefore some sera were retested. We used the results from the first test when calculating sensitivity and the second result if available for calculation of the specificity. Almost all sera from non-infected cattle that scored positive (Ceditest 21 out of 1 100) were retested (for the Ceditest only 3 sera from non-infected cattle that were positive in the first test were not retested). For both the non-infected as well as the infected cattle we analysed if there was difference in results between non-vaccinated and vaccinated cattle. In the non-infected cattle we found no difference in the results of the Ceditest between non-vaccinated and vaccinated cattle (neither in the t-test on the actual percentage inhibition, neither by testing positive negative results in a Chi Square test). Based on the results obtained in the workshop an overall specificity of 99.2% (95% CI: 98.5 - 99.6%) was estimated.

In the samples from exposed cattle, there was a significant difference between the positive and negative results obtained from non-vaccinated and vaccinated cattle. In the non-vaccinated cattle the overall sensitivity was 98.2% (95% CI: 90.3 - 100%). In the vaccinated cattle the overall sensitivity was 60.4% (95% CI: 54.1 - 66.4%). Within the population of vaccinated and exposed cattle large differences were seen between various selections e.g. in vaccinated and exposed cattle where serum was collected between 28 - 100 days (n = 92) after exposure the sensitivity was 59.8%, if cattle was selected in which infection was confirmed the sensitivity in this group (n = 76) became 65.8%, and if only carrier cattle were selected the sensitivity became 88% (n = 50). For the calculation of the sample size we used a worst-case sensitivity of 55%. In an outbreak situation the sensitivity of the test will most probably be higher than the values found in the validation study, because in animal experiments the animals are always correctly vaccinated, but after an emergency vaccination the response to vaccination will be lower. More animals will react like the non-vaccinated cattle exposed in animal experiments (overall sensitivity 98.2%).

Number of samples per farm

Based on the formula given by Martin et al. the average number of samples that would have been collected in the 2 km areas surrounding the 2001 outbreak farms, was 41(this would be 30 samples if a test with 95% sensitivity was used). In total 39 701 samples would have been collected on 972 farms. The total number of samples collected based on the 95% upper limit from the IRMA report "Scenario-onderzoek effectiviteit vaccinatie en impact op afzet producten" would be 1 210 x 41 = 49 610 samples in total. In this case we assumed that the farm size in the area used in the report is similar to the farm size in the area where the 2001 outbreak occurred. This assumption is likely to be correct because 24 of the 26 outbreaks in 2001 occurred in the most densely populated livestock area described in the report.

Simulation of false positive samples

From the farms that would have been sampled if in 2001 emergency vaccination in a 2 km radius was applied, on average 247 farms would have had a positive result. The distribution of the number of positive farms is shown in figure 3. In 1000 simulations the number of farms that had to be retested ranged from 214 to 288.

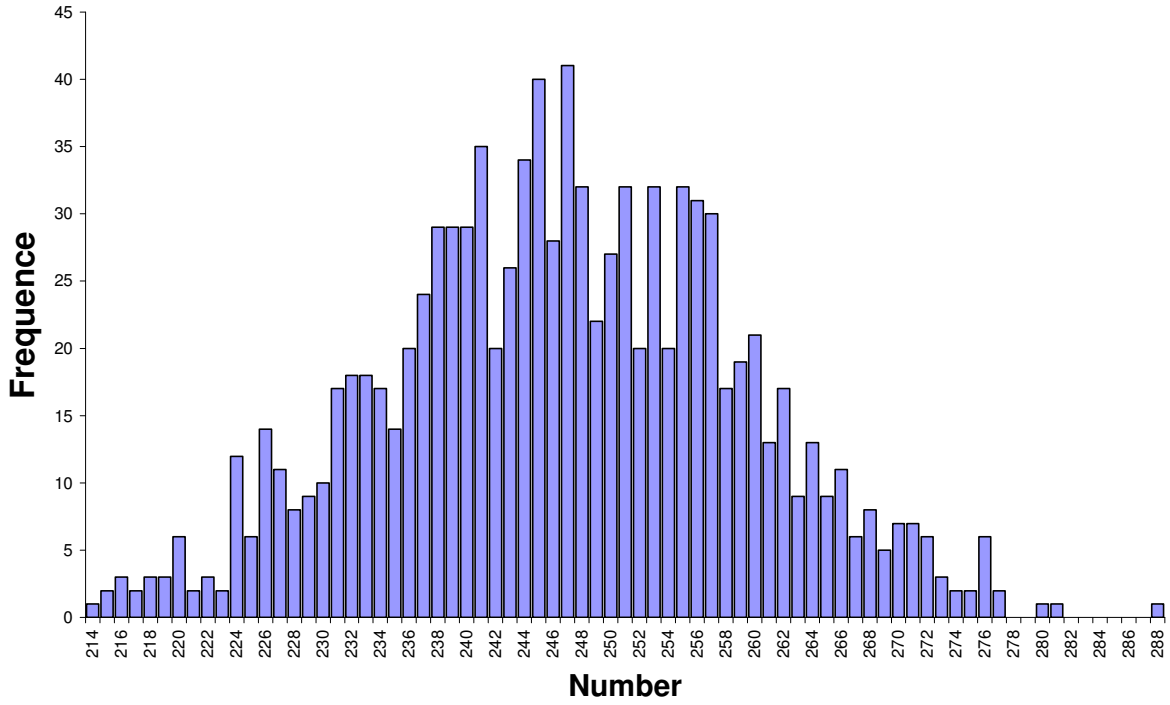


Figure 3: Distribution of the number of farms with a false positive result (based on 1000 simulations)

In total 13 908 to 18 145 samples (average 16 069 / 247 = 66 samples per farm) would have been collected on these farms for the first retesting. In 1000 simulations on average 97 farms would again have one or more positive samples in the second retest (range 71 - 126). The total number of samples in the second retest would range from 5 304 to 9 548 with an average of 7 335.

Using the numbers from the report "Scenario-onderzoek effectiviteit vaccinatie en impact op afzet producten" the number of positive farms would be 247 / 972 X 1 210 = 308. Resulting in 66 x 308 = 20 328 samples in the first retest (maximum 1 210 / 972 X 18 145 = 22 588). On average 1 210 / 972 X 97 = 121 farms would turn up positive again, resulting in 1 210 / 972 X 7 335 = 9 131 positive samples (maximum 1 210 / 972 X 9 548 = 11 886).

Discussion

The purpose of this report is to estimate the numbers of samples that likely have to be tested in the aftermath of an FMD outbreak in the Netherlands, if emergency ring vaccination in a 2 km radius is performed. To derive estimates on the average number of samples per farm and the average number of farms that have to be retested we used detailed data from the FMD outbreaks in 2001. This can be considered as a small to average size of FMD outbreak. To estimate the numbers of samples that have to be tested in a large outbreak we used the previous estimates with the 95% estimates of number of vaccinated farms and number of vaccinated animals from the report "Scenario-onderzoek effectiviteit vaccinatie en impact op afzet producten" (IRMA September 2004).
In 2001 972 farms would have been vaccinated if a 2 km ring vaccination strategy would have been deployed. When sampling these farms with 95% confidence to detect a 5% prevalence of antibodies against non-structural proteins with a test with 55% sensitivity this would result in 39 701 samples for initial screening. Even if all samples would be truly negative, a test with

99.2% specificity would still on average result in 247 positive farms. If these farms were completely sampled this would on average result in 16 069 additional samples. Completely resampling would result in more (false) positive results, and probably another round with a maximum of 10 000 samples. In total less than 65 000 samples for screening of the vaccinated areas, whereas in the 2001 outbreak almost 200 000 samples were tested. However, if vaccination is used also samples from the protection and surveillance zone will have to be tested. The samples collected from the vaccinated will therefore be supplemental to the other samples collected. But the total number is not expected to be higher than 265 000 samples.

In the case of a large outbreak (95% upper limit IRMA report), up to 85 000 samples have to be tested (screening and maximum numbers of first and second retest) and on average 308 farms will be blocked because of positive results in the screening. After 2 times retesting 121 farms will still have positive serological results. Epidemiological evaluation of the results and positive contacts will define the status of these farms.

The proposed sampling tree still has no solution if one or more positive samples are found several times on the same farm, but no clustering of the positive samples is detected. This will happen on average on 133 farms, if no clustering is found these farms should be considered free of FMD infection. In discussion in Brussels this issue has been discussed with Alf Fuessel, who also agreed that only farms where FMD is present should be culled. Farms with only serological positive results should be epidemiologically evaluated

The numbers of samples that have to be collected and tested do not exceed the capacity for sampling and testing (in previous outbreaks over 50 000 serum samples per week were collected and tested). According to § 56 of EU/2003/85 sampling should not start earlier than 30 days from the date of completion of emergency vaccination. To be able to lift the control measures within 3 months, only 2 months are left for screening and resampling of herds. The first screening should be finished within 3 weeks to be able to do the resampling and retesting in the remaining time. The time needed for collection of the samples will not exceed much the time which was necessary in 2001. In 2001 the final screening started on 2 May and was finished 4 weeks later on 31 May, totally 47 496 sera were examined during this final screening. So not the laboratory capacity but the capacity to sample animals might be the limiting factor. Contingency plans of both laboratory and the one of the inspection service of livestock and meat should address this issue. When the screening has to be performed within a smaller timeframe more resources should be made available for the teams in the field, but probably also for the laboratory.

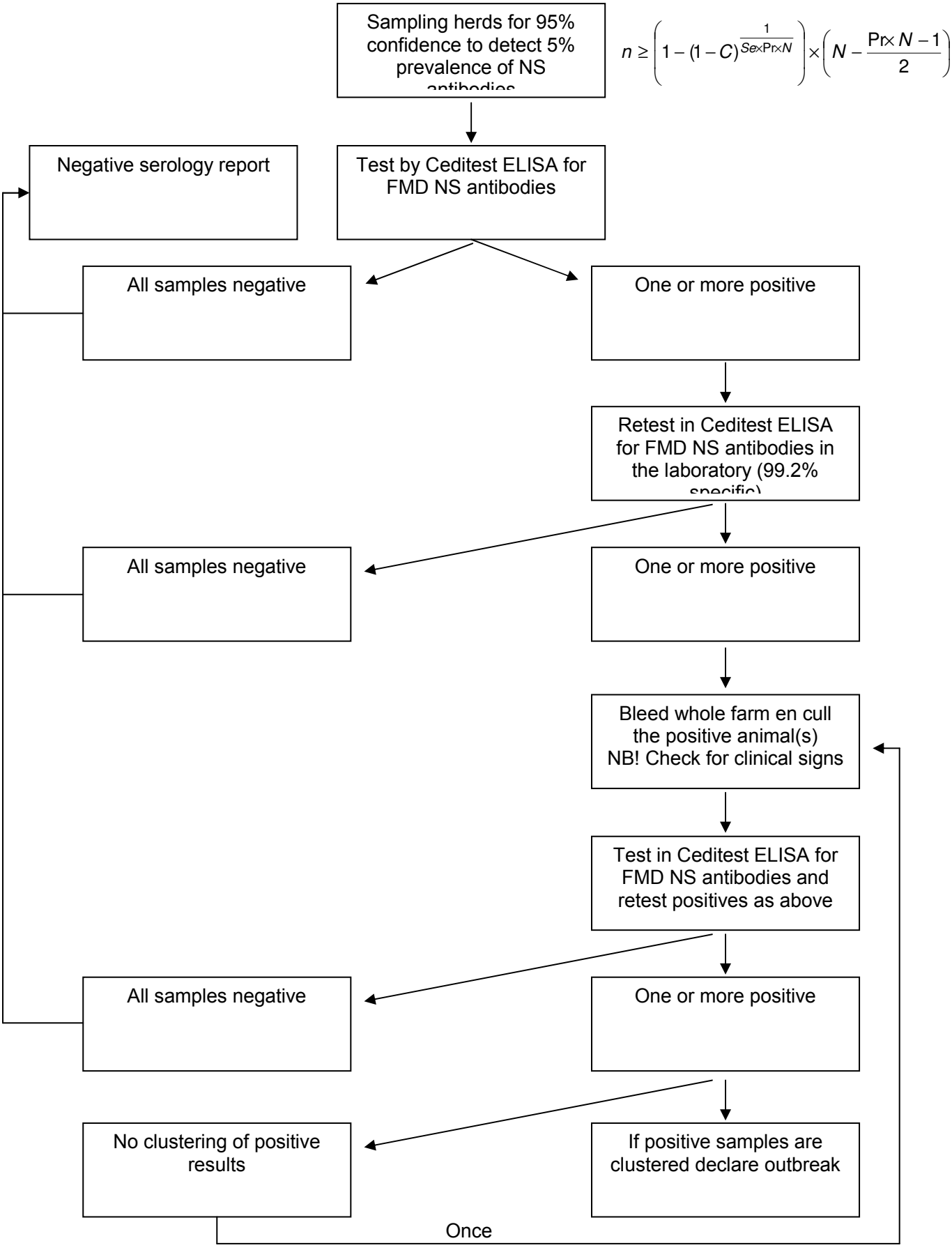
Some issues have to be resolved:

- What do we do with repeated positive findings in a herd without clustering?
    - A decision tree like the one in chapter VI of decision 2000/428/EC for SVD singleton reactors would be justifiable, with the modification that for pigs susceptible animals is read and only from susceptible ruminants probang samples are collected instead of faecal samples.
- How do we sample large pig fattening farms at resampling? Testing the whole farm doesn't seem necessary, because FMD spreads very quickly within a pen, so 2 samples per pen should be adequate.
- Will titratation of false positive samples in the Ceditest ELISA give additional information on true or false positivity?
- The effect of retesting positive samples in another ELISA for FMD non-structural proteins on sensitivity and specificity should be addressed.

References

Martin, S. W., Shroukri, M., Thorburn, M. A. 1992. Evaluating the heath status of herds based on tests applied to individuals. Preventive Veterinary Medicine 14: 33-43.

Appendix A. Sampling tree



Sero-monitoring for FMD infection in the Trans-Caucasus

Carsten J. Pötzsch
- FLI, Germany -

Acknowledgements

Christoph Staubach, FLI
Andriy Rozstalnyy, FAO
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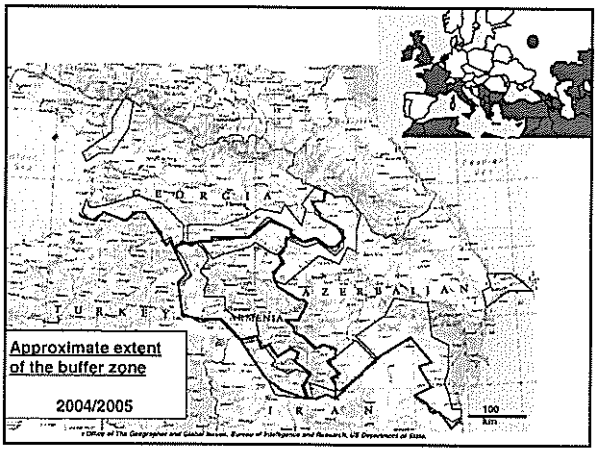
Emiliana Brocchi, NRL; Italy
Keith Sumption , FAO

Design of the sero-monitoring

- Sampling type: stratified random sampling
- Epidemiological unit: cattle population of a village
- detection of 10% inter-herd and 10% intra-herd prevalence (NSP) at 95% confidence
- Focus on animals ≤2yrs
- Study area: buffer zone

Questionnaire design

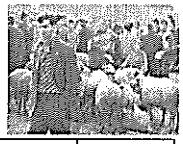
1. origin of animal: rayon, village
2. owner
3. species: cattle, buffalo
4. age: month, year (or teeth)
5. sex
6. previous FMD vaccinations
7. previous FMD disease of this animal
8. previous FMD disease in the herd



FMD outbreaks, 1998 - 2004

	Georgia	Armenia	Azerbaijan	Turkey	Iran
1998	A	A	-	O, A	O, A
1999	A	A	-	O, A, Asia1	O, A, Asia1
2000	O, Asia1	O, Asia1	-	O, A, Asia1	O, A, Asia1
2001	O, Asia1	-	Asia1	O, A, Asia1	O, A, Asia1
2002	O	O	-	O, A, Asia1	O, A, Asia1
2003	-	-	-	O, A	O, A
2004	-	-	-		O, A, Asia1

Livestock numbers



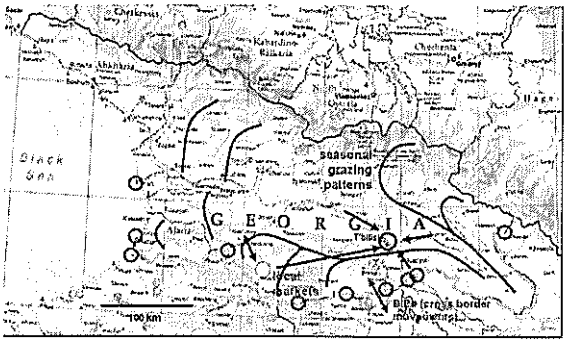
	Georgia	Armenia	Azerbaidshan	total
• cattle	1.11 mill.	640,000	2,24 mill.	4 mill.
• small ruminants	670,000	800,000	7.28 mill.	8.8 mill.
• pigs	470,000	150,000	-	0.6 mill.
• camels	-	-	2,000	

Livestock systems

⇒ village = epidemiological unit
small farms, mixed species

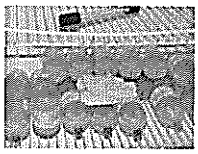
extensive regional movements of animals and products
- seasonal grazing
- local livestock markets

Major livestock movements

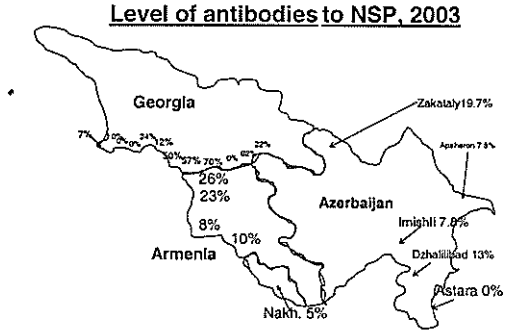


Vaccine use

1. Vaccination was apparently carried out as agreed
2. Storage conditions/ cold chain need improvement



Level of antibodies to NSP, 2003



Armenia, sampling sites

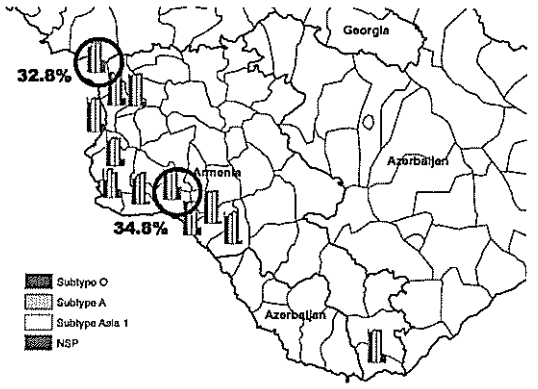
Armenia, age distribution, rayons

Armenia: SP and NSP ELISA results

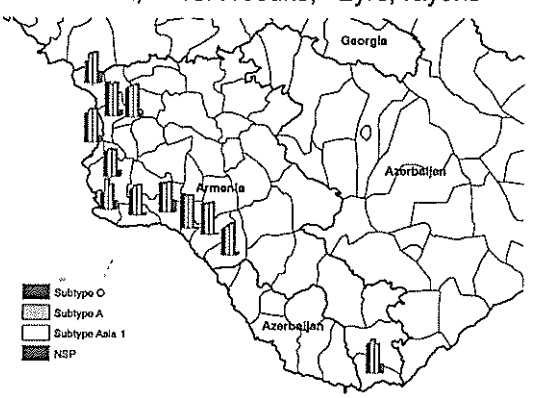
Subtype		
• O	82.8%	50-100%
• A	91.5%	63.3-100%
• Asia1	93.1%	53.3-100%
3ABC	14.5%	0-86.7%

(range on village level)

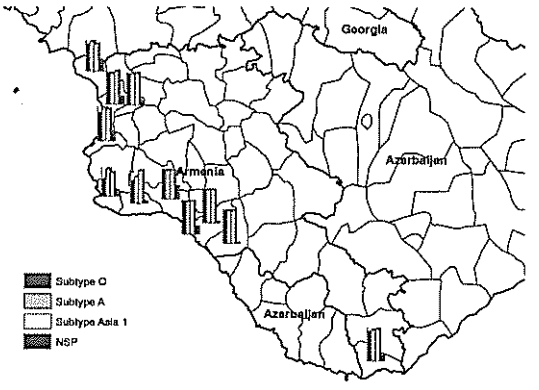
Armenia, ELISA results, rayons



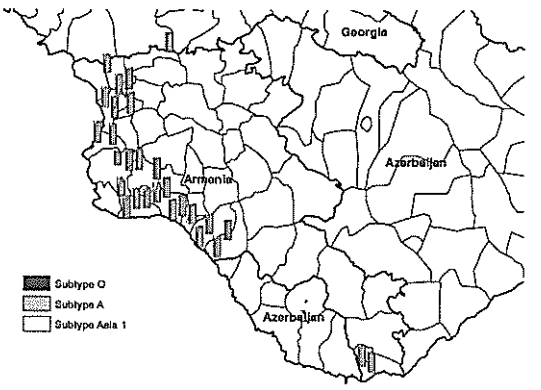
Armenia, ELISA results, ≤2yrs, rayons



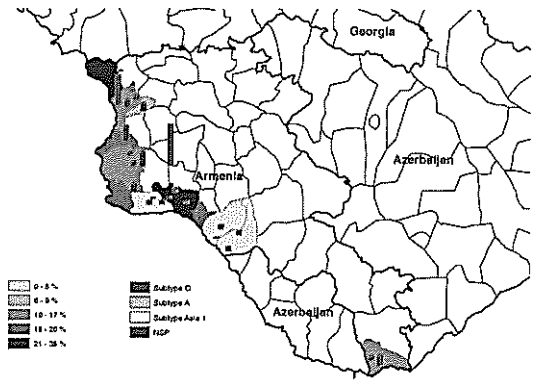
Armenia, ELISA results, >2yrs, rayons



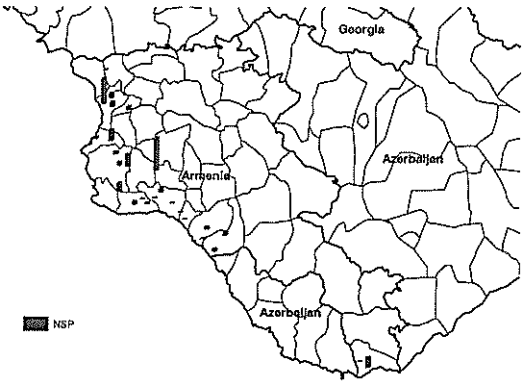
Armenia, SP results, villages



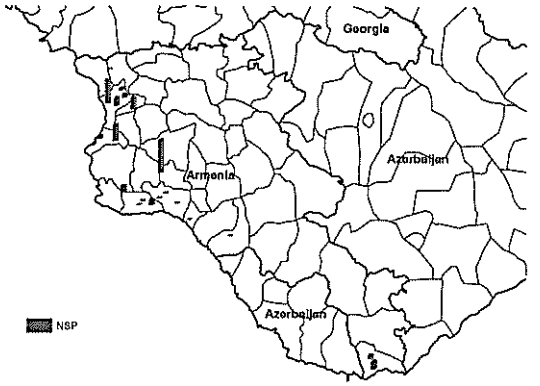
Armenia, NSP results, villages



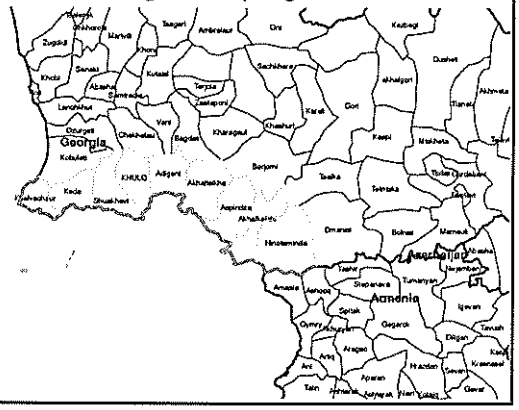
Armenia, NSP results, ≤2yrs, villages



Armenia, NSP results, >2yrs, villages



Georgia, sampling sites

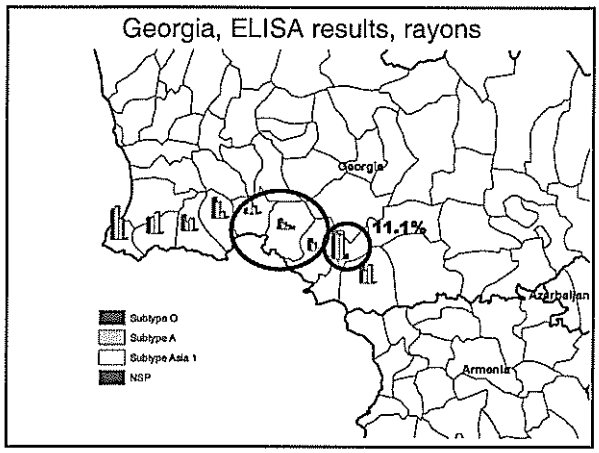


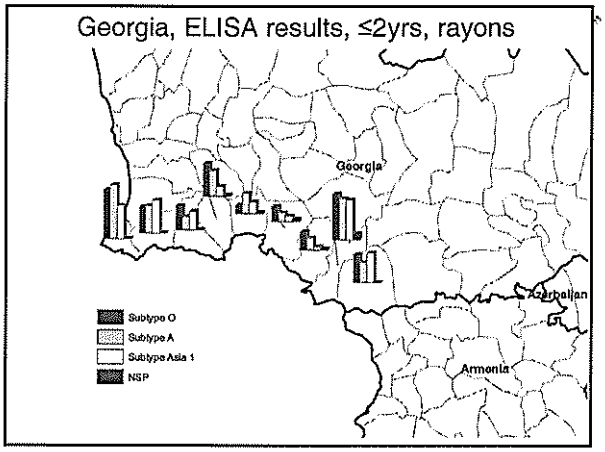
Georgia, age distribution, rayons

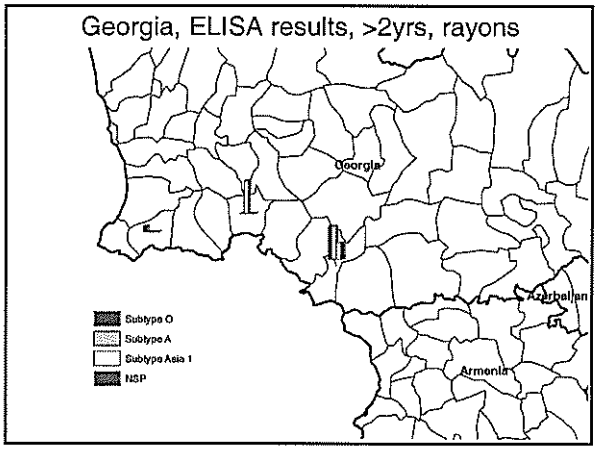
Georgia: SP and NSP ELISA results

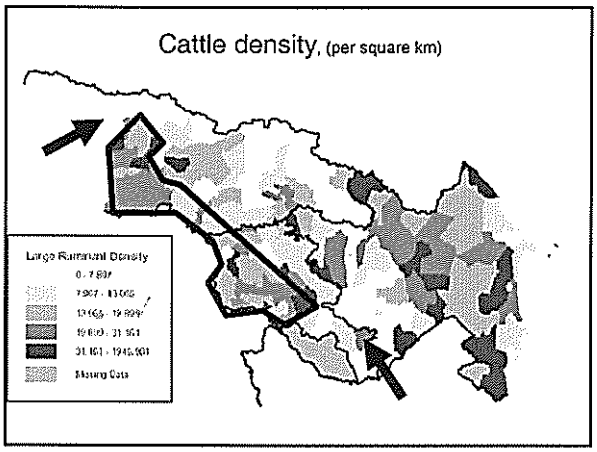
Subtype		
• O	46.6%	14.4-80.7%
• A	41.4%	14.4-88.2%
• Asia1	34.5%	5.6-65.6%
3ABC	2.5%	0-11.1%

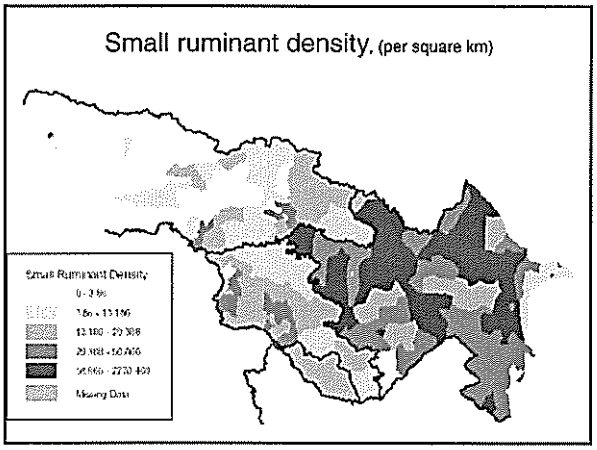
(range on rayon level)

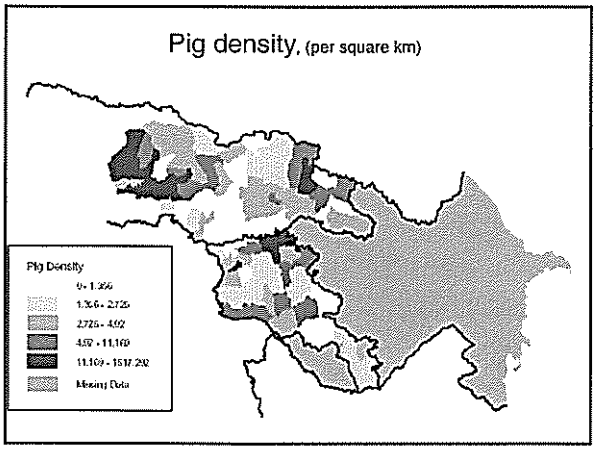

Georgia, ELISA results, rayons
Georgia
11.1%
Azerbaijan
Armenia
Subtype O
Subtype A
Subtype Asia 1
NSP


Georgia, ELISA results, ≤2yrs, rayons
Georgia
Azerbaijan
Armenia
Subtype O
Subtype A
Subtype Asia 1
NSP


Georgia, ELISA results, >2yrs, rayons
Georgia
Azerbaijan
Armenia
Subtype O
Subtype A
Subtype Asia 1
NSP


Cattle density, (per square km)
Large Ruminant Density
Missing Data


Small ruminant density, (per square km)
Missing Data


Pig density, (per square km)
Pig Density
Missing Data

Conclusions

- No proof of circulation of FMD
- High NSP/high SP in Armenia:
  - vaccine effect or disease history ?
  - Animal movement [(cross-border) trade] ?
  - ?
- High variation in level of NSP prevalences (Armenia) and SP prevalences (Georgia)
- No distinct age effect on SP and NSP results
- Effect of study design and execution (selection of animals) ?

Outlook

- Continue analysis
- Baseline study, include:
  - Individual animal information (vaccination history)
  - sheep, pigs
  - Narorgny Karabakh, Abkhasia
- Trade flows and market studies
- Regional cooperation
- Re-evaluate the concept of the buffer zone

Overview of results of serosurvey in Transcaucasian region - buffer zones

Emiliana Brocchi
Santina Grazioli

FMD NRL
Istituto Zooprofilattico Sperimentale
Brescia - Italy

IZSLER BRESCIA

Serosurvey in Transcaucasian region

Objectives

- **Vaccinal immunity**
  - **level and distribution of antibody to SP**
- **Investigate on possible virus circulation**
  - **antibodies to NSP**

Country	N° Villages / N° Counties	Sampling dates	N° sera	conditions
ARMENIA	30 / 12	16-17 June	**900**	Good
GEORGIA	31 / 9	11-13 July	**929**	Very good
AZERBAIJAN	30 / 8	6-7 June	**948**	Bad (leaking)

Arrival Brescia → end July

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TESTS

Tests	Sampling dates	Serum dilut	NOTES
O Manisa	LPB-ELISA	1/10→1/2430	In-house Mabs-based Recognise correctly RS
A Iran 96	SPC-ELISA	1/10→1/2430	
Asia 1	SPC-ELISA	1/10→1/2430	
NSP-3ABC	3ABC-ELISA	1/100	POS retested by IZS-Brescia & Ceditest

Sera treated 56 °C, 45 minutes

- ✓ Testing conducted during August
  - holidays time, less human resources, less routine serology
- ✓ 15 full (wet) working days
  - 60 plates/day SP ELISAs or 20 plates/day NSP-ELISA
- ✓ Data entry & elaboration

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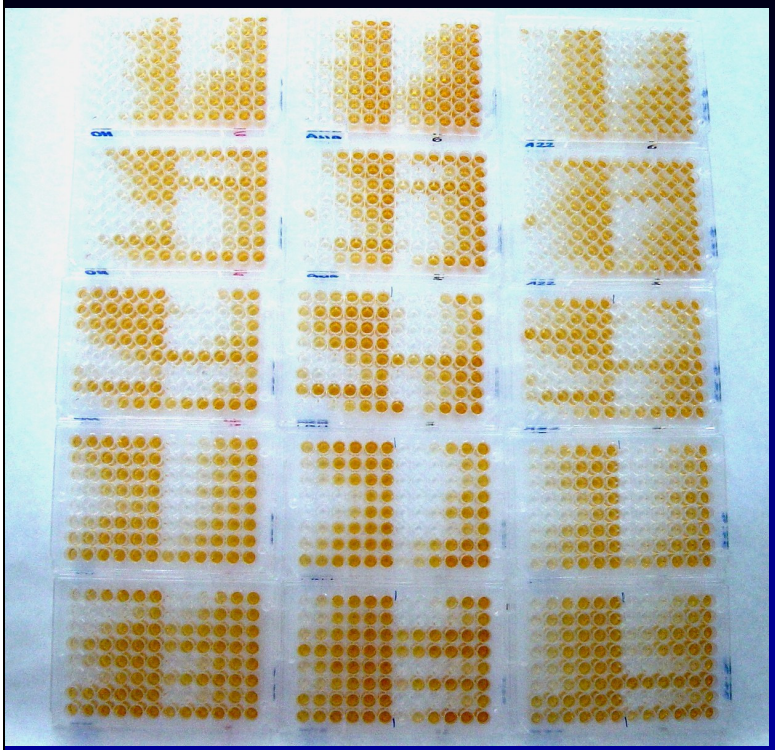
O Manisa Asia 1 A Iran 96

SP-ELISA



Serum dilution: range 1/10 → 1/2430

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ARMENIA

Region	County	Town, village	N° cattle	age range years	NSP-Ab Pos	NSP-Ab % Pos
Ararat	Ararat	Vedi	30	2	2	6,7
		Surenavan	30	1-2	2	6,7
		Shagap	30	1-2	1	3,3
		Avshar	30	1-2	0	0
	Masis	Hovtashat	30	3-7	11	36,7
		Darbnik	30	1-9	0	0
	Artashat	Berkhanush	30	1-3	0	0
		Mxchyan	30	1-2	0	0
Armavir	Echmiadzin	Akhavnatun	30	1-3	28	93,3
		Lernamerc	30	1-3	3	10
		Echmiadzin t.	30	1-3	2	6,7
	Armavir	V. Armavir	30	1-3	2	6,7
		Eghegnut	31	1-3	2	6,5
		Lenughi	30	1-3	0	0
		Arevik	29	1-3	0	0
	Bagramyan	Myasnikyan	30	1-4	6	20
Aragacotn	Talin	N.Sasnashen	30	1	10	33,3
		Davtashen	30	1-2	3	10
		Mastara	30	1-2	2	6,7
Syuniq	Meghri	Meghri	28	1-10	7	25,0
		Lehbaz	30	3-7	3	10
Shirak	Amasia	Vogchi	30	1-7	19	63,3
		Alvar	28	1-5	0	0
	Ani	Qarabert	30	1-6	10	33,3
		Jrapi	30	1-4	2	6,7
	Akhuryan	Musaelyan	30	1-4	5	16,7
		Maisyan	30	2-7	3	10
		Sariar	30	1-9	2	6,7
		Pograshen	30	1-7	1	3,3
	Gyumry	Gyumry	30	1-5	5	16,7

Overall 131 sera/896
POS NSP-Ab (15 %)

N° 9/30 villages
(all regions)
High seroprevalence
17% → 93% (clusters)

N° 14/30 villages
1 → 3 seropositive/vill.
3,3% → 10%
lower virus circulation?
animal movement?

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ARMENIA

Region	County	Town, village	N° cattle	age range years	NSP-Ab Pos	NSP-Ab % Pos	O Manisa % Pos	A Iran 96 % Pos	Asia 1 % Pos
Ararat	Ararat	Vedi	30	2	2	6,7	73	73	93
		Surenavan	30	1-2	2	6,7	83	90	100
		Shagap	30	1-2	1	3,3	93	93	93
		Avshar	30	1-2	0	0	70	100	100
	Masis	Hovtashat	30	3-7	11	36,7	100	100	100
		Darbnik	30	1-9	0	0	97	100	100
	Artashat	Berkhanush	30	1-3	0	0	90	90	93
		Mxchyan	30	1-2	0	0	100	100	100
Armavir	Echmiadzin	Akhavnatun	30	1-3	28	93,3	100	100	100
		Lernamerc	30	1-3	3	10	70	70	80
		Echmiadzin t.	30	1-3	2	6,7	93	93	93
	Armavir	V. Armavir	30	1-3	2	6,7	90	97	87
		Eghegnut	31	1-3	2	6,5	81	90	81
		Lenughi	30	1-3	0	0	53	67	90
		Arevik	29	1-3	0	0	76	97	100
	Bagramyan	Myasnikyan	30	1-4	6	20	50	87	73
Aragacotn	Talin	N.Sasnashen	30	1	10	33,3	87	90	90
		Davtashen	30	1-2	3	10	77	100	100
		Mastara	30	1-2	2	6,7	60	63	53
Syuniq	Meghri	Meghri	28	1-10	7	25,0	89	96	89
		Lehbaz	30	3-7	3	10	87	97	100
Shirak	Amasia	Vogchi	30	1-7	19	63,3	87	93	93
		Alvar	28	1-5	0	0	57	86	82
	Ani	Qarabert	30	1-6	10	33,3	83	100	97
		Jrapi	30	1-4	2	6,7	87	100	100
	Akhuryan	Musaelyan	30	1-4	5	16,7	90	97	100
		Maisyan	30	2-7	3	10	93	100	100
		Sariar	30	1-9	2	6,7	90	90	93
		Pograshen	30	1-7	1	3,3	83	93	97
	Gyumry	Gyumry	30	1-5	5	16,7	97	93	100
TOTAL / AVERAGE %			896		131	15 %	83 %	92 %	93 %

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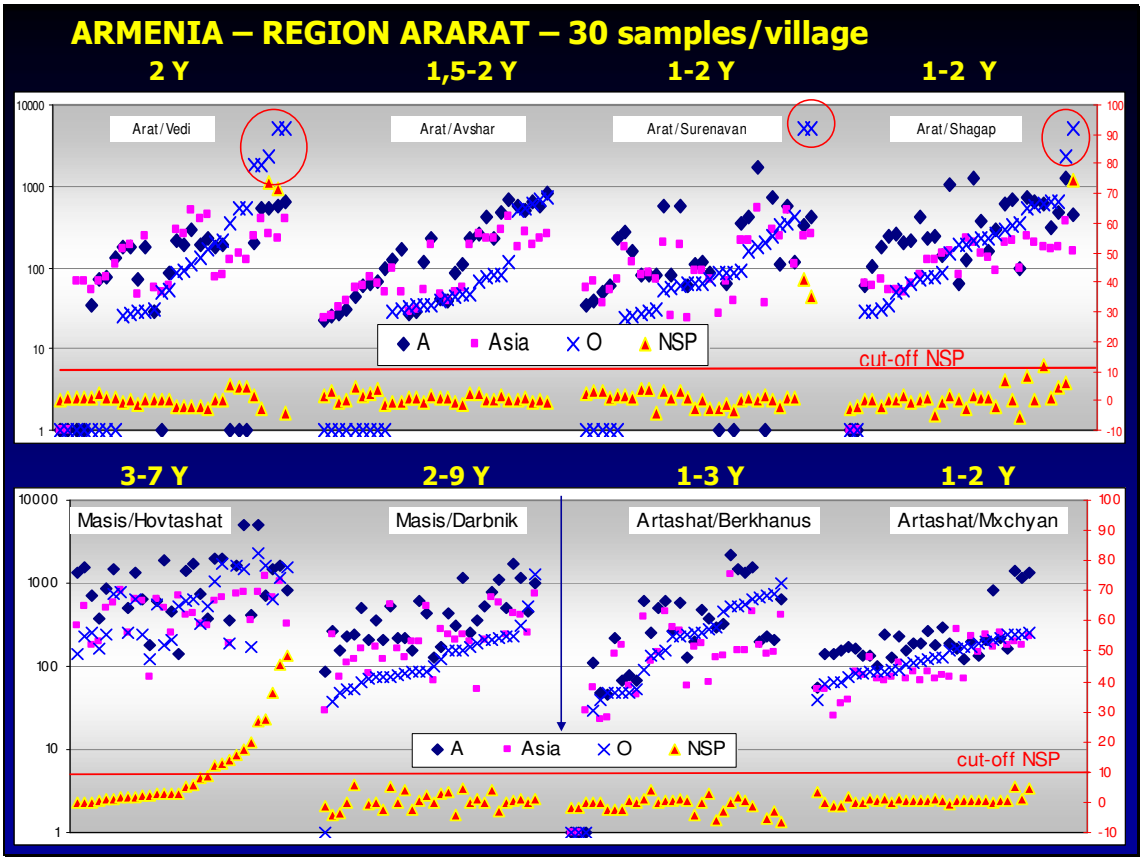
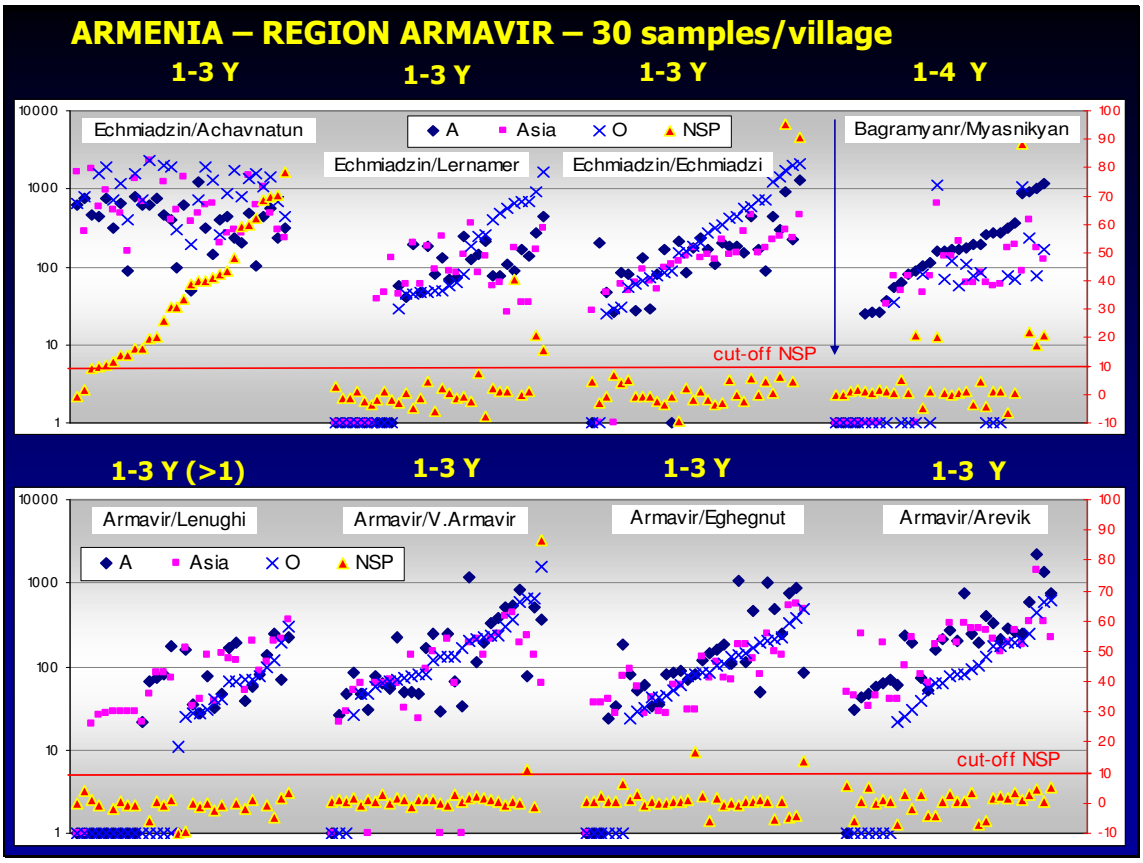
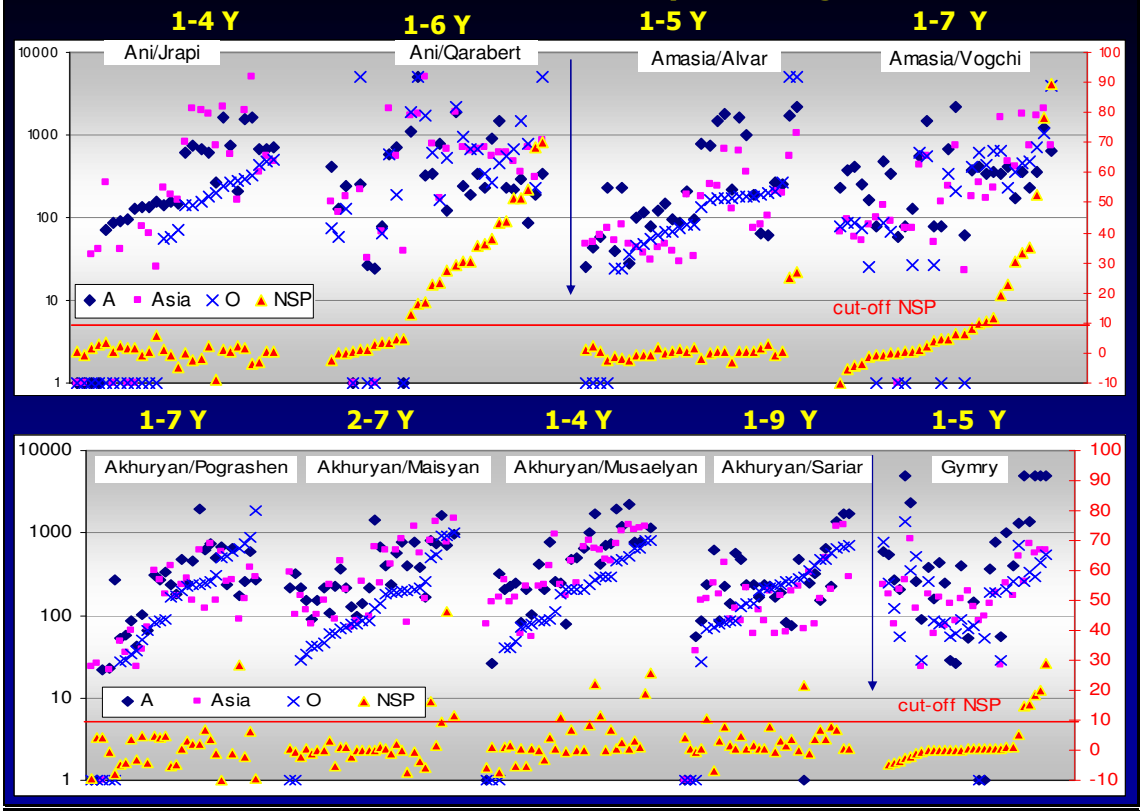
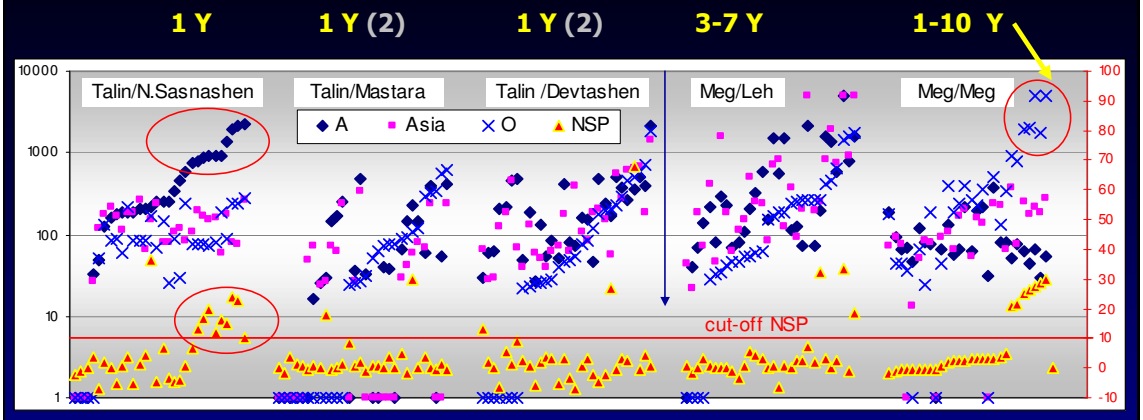

ARMENIA – REGION ARARAT – 30 samples/village
2 Y
1,5-2 Y
1-2 Y
1-2 Y
Arat/Vedi
Arat/Avshar
Arat/Surenavan
Arat/Shagap
A
Asia
O
NSP
cut-off NSP
3-7 Y
2-9 Y
1-3 Y
1-2 Y
Masis/Hovtashat
Masis/Darbnik
Artashat/Berkhanus
Artashat/Mxchyan
A
Asia
O
NSP
cut-off NSP


ARMENIA – REGION ARMAVIR – 30 samples/village
1-3 Y
1-3 Y
1-3 Y
1-4 Y
Echmiadzin/Achavnatun
Echmiadzin/Lernamer
Echmiadzin/Echmiadzi
Bagramyanr/Myasnikyan
A
Asia
O
NSP
cut-off NSP
1-3 Y (>1)
1-3 Y
1-3 Y
1-3 Y
Armavir/Lenughi
Armavir/V.Armavir
Armavir/Eghegnut
Armavir/Arevik
A
Asia
O
NSP
cut-off NSP

ARMENIA – REGION SHIRAK– 30 samples/village



ARMENIA – REGIONS ARAGACOTN, SYUNIQ - 30 samples/village



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GEORGIA

County	Town, village	N° cattle	age range years	NSP-Ab Pos	NSP-Ab % Pos
Shuakevi	Gogadzeebi	30	1 - 2 years	1	3,3
	Vani	30		0	0
	Nenia	30		0	0
	Brili	30		0	0
Ninotsminda	Dilipi	30		0	0
	Udjmana	30		0	0
	Spasovka	30		0	0
Khulo	Ganakhleba	30		3	10
	Vernebi	30		0	0
	Gurta	30		0	0
	Bodzauri	30		0	0
Aspindza	Atskvita	30		0	0
	Saxudabeli	30		2	6,7
	Tmogvi	30		0	0
Akhalkalaki	Sulda	30		10	33,3
	Alastini	30		0	0
	Khorenia	30		0	0
Khelvachauri	Zanakidzeebi	30		1	3,3
	Shua makhinjauri	29		0	0
	Ortabatumi	30		0	0
	Kveda chkhutuneti	30		0	0
Akhaltsikhe	Kulalisi	30		2	6,7
	Sviri	30		2	6,7
	Zikilia	30		0	0
Adigeni	Kikibo	30		1	3,3
	Patara Zanavi	30		1	3,3
	Chorchali	30		0	0
Keda	Zeda agara	30		0	0
	Tsoniaresi	30		0	0
	Koromkheti	30		0	0
	Dzesopeli	30		0	0

Overall 23 sera/929
POS NSP-Ab → 2.5 %

N° 1/31 villages
High seroprevalence
33.3 % (cluster)

N° 8/31 villages
3seropositive/vill.
3,3% → 6.7%
low virus circulation?
animal movement?
false-positive? (1.4 %)

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GEORGIA

County	Town, village	N° cattle	age range years	NSP-Ab Pos	NSP-Ab % Pos	O Manisa % Pos	A Iran 96 % Pos	Asia 1 % Pos
Shuakevi	Gogadzeebi	30	1 - 2 years	1	3,3	50	37	40
	Vani	30		0	0	17	0	17
	Nenia	30		0	0	77	40	57
	Brili	30		0	0	17	10	10
Ninotsminda	Dilipi	30		0	0	37	33	50
	Udjmana	30		0	0	40	30	37
	Spasovka	30		0	0	57	33	57
Khulo	Ganakhleba	30		3	10	70	80	33
	Vernebi	30		0	0	50	20	0
	Gurta	30		0	0	67	63	20
	Bodzauri	30		0	0	30	3	10
Aspindza	Atskvita	30		0	0	17	3	3
	Saxudabeli	30		2	6,7	63	40	13
	Tmogvi	30		0	0	20	17	0
Akhalkalaki	Sulda	30		10	33,3	87	63	67
	Alastini	30		0	0	67	67	63
	Khorenia	30		0	0	73	73	67
Khelvachauri	Zanakidzeebi	30		1	3,3	63	60	27
	Shua makhinjauri	29		0	0	66	100	83
	Ortabatumi	30		0	0	93	100	97
	Kveda chkhutuneti	30		0	0	100	93	13
Akhaltsikhe	Kulalisi	30		2	6,7	30	20	10
	Sviri	30		2	6,7	17	7	10
	Zikilia	30		0	0	30	17	7
Adigeni	Kikibo	30		1	3,3	23	33	23
	Patara Zanavi	30		1	3,3	10	23	17
	Chorchali	30		0	0	13	47	17
Keda	Zeda agara	30		0	0	17	40	17
	Tsoniaresi	30		0	0	7	0	100
	Koromkheti	30		0	0	60	63	50
	Dzesopeli	30		0	0	80	67	57
TOTAL / AVERAGE		929		23	2.5 %	47 %	41 %	34 %

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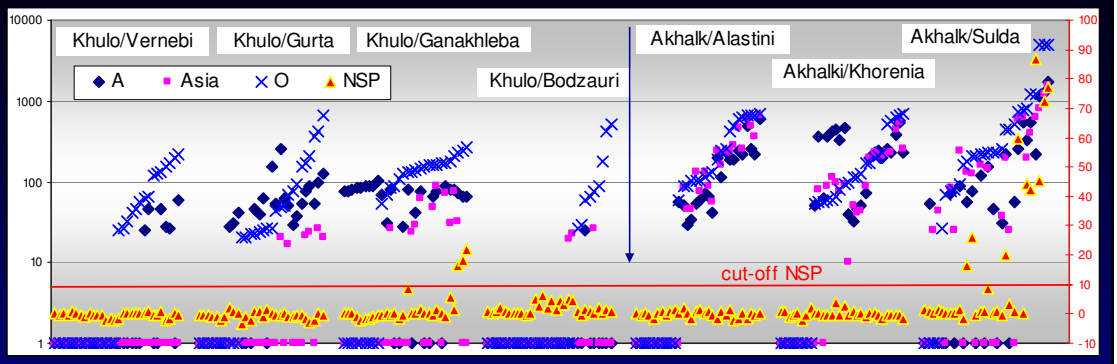
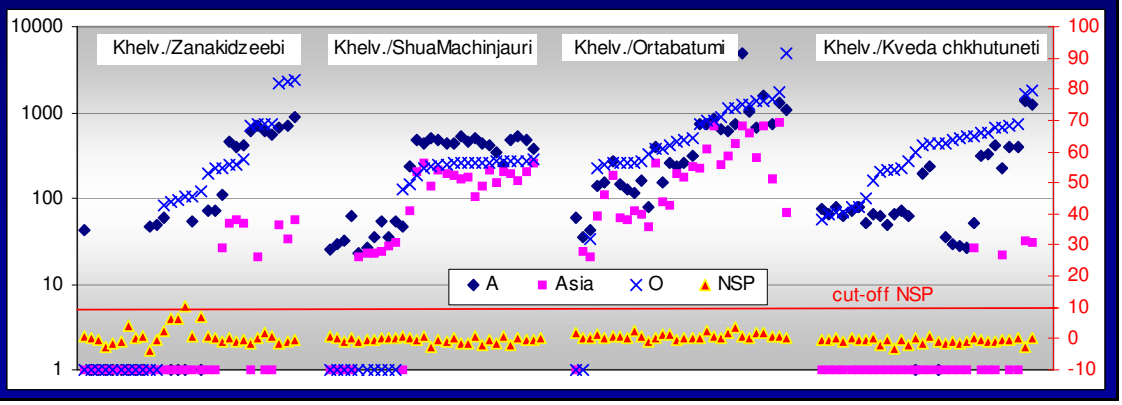
GEORGIA - ANIMALS AGE 1 – 2 YEARS – 30 SAMPLES/VILLAGE

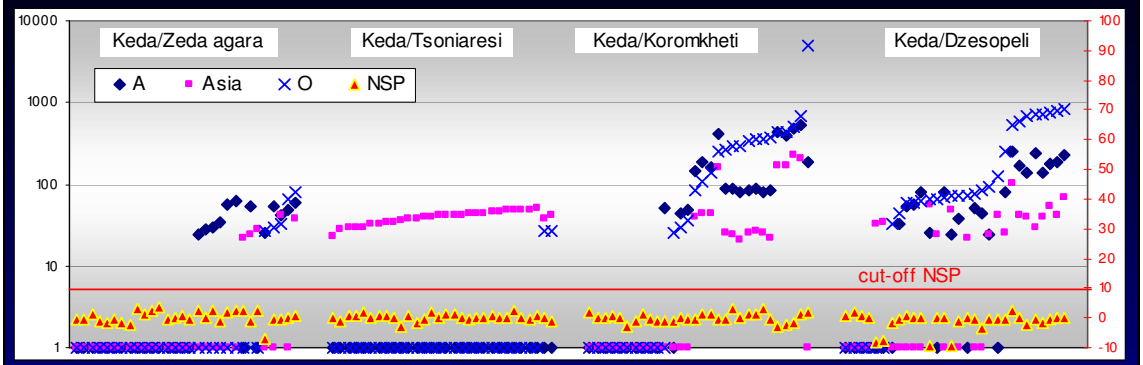


↑ REGIONS KHULO, ALKHALSIKHE ↓ REGION KHELVACHAURI



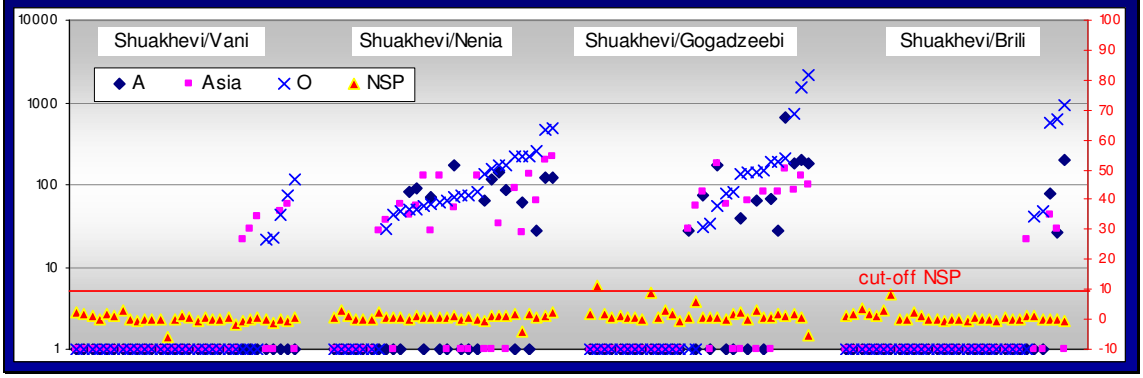
REGION KEDA

GEORGIA - CATTLE AGE 1 – 2 YEARS

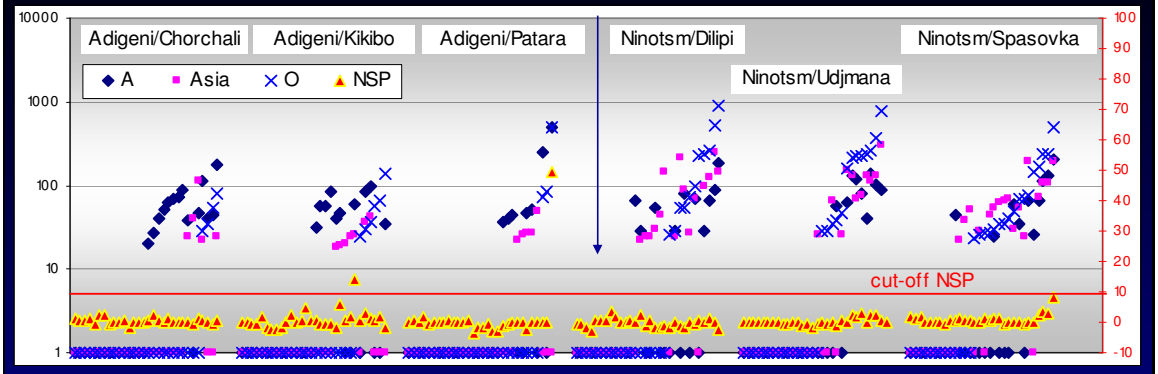


REGION SHUAKEVI

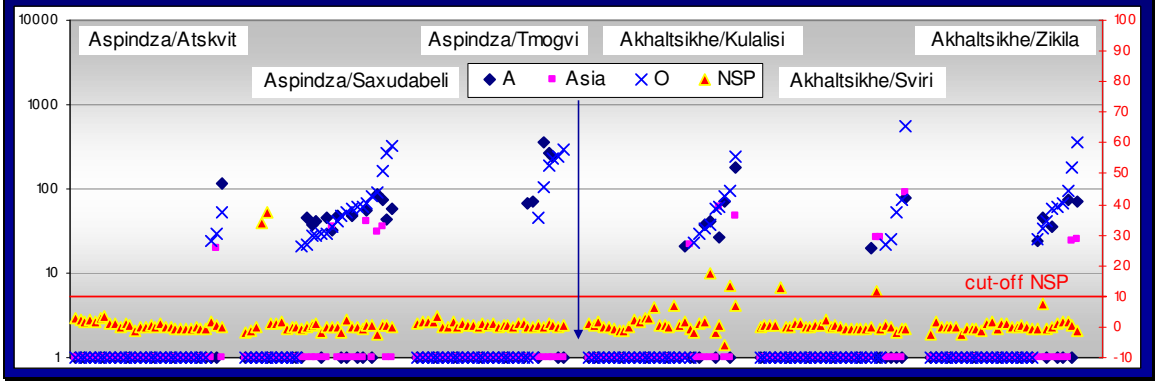
30 samples/village



REGION ADIGENI, NINOTSMINDA — GEORGIA



REGION ASPINDZA, AKHALTSIKHE — 30 samples/village, age 1-2 years



AZERBAIJAN

County	Town, village	N° cattle	age range years	NSP-ELISA Pos	NSP-ELISA % Pos
Astara	Anbabiu	47	1-2	3	6,4
	Motalayag	14	1-8	0	0
	Tahtakaran	35	1-3	0	0
Lerik	Almew	32	0.3-1.2	0	0
	Kokoniu	26	1-2	0	0
	Arta	15	1-2	0	0
Beyliagan	Orengala	28	0.5-1.3	0	0
	Tiurkler	33	0.5-1.5	0	0
	Khachynabad	32	0.6-1.6	0	0
	Gadekler	31	0.8-1.2	0	0
Belasuar	Fioletovka	32	0.9-4	8	25
	Tazakend	34	2-4	10	29,4
	Arkhangelovka	28	1-2	1	4
Imishli	Jeferly	26	0.5-1.3	5	19
	YukharyGaragiuvendly	33	0.6-1.8	3	9,1
	Khelfaly	34	1-1.9	2	6
	Sarykhanly	34	0.3-2	2	6
	Badgyravan	27	0.8-1.9	0	0
Jalilabad	Khalilly	30	1-2	9	30
	Khajejavadly	33	1-2	4	12,1
	Laken	29	1-3	3	10,3
	Khoviuzbiulag	30	1-2	2	6,7
	Adnaliu	48	0.7-2	2	4,2
	Biuravar	21	1-2.5	1	4,8
	Shykhlar	28	1-2	1	3,6
	Lezran	30	1-2	0	0
Fyziuli	Alhanbeyly	39	1-5	16	41
	Kerimbeyly	39	1-7	4	10
Yardimly	Unash	34	0.5-2	2	6
	Avin	39	0.7-2	1	2,6

Overall 79 sera/939
POS NSP-Ab → 8.4 %

N° 5/30 villages
(4 counties)
High seroprevalence
19% → 41% (clusters)

N° 14/30 villages
1 → 4 seropositive/vill.
2.6% → 12%
lower virus circulation?
animal movement?

N° 2 counties
NO virus circulation

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AZERBAIJAN

County	Town, village	Nº cattle	age range years	NSP-ELISA		O Manisa	A Iran 96	Asia 1
				Pos	% Pos	% Pos	% Pos	% Pos
Astara	Anbabiu	47	1-2	**3**	**6,4**	89	98	100
	Motalayag	14	1-8	0	0	64	93	100
	Tahtakaran	35	1-3	0	0	91	91	51
Lerik	Almew	32	0.3-1.2	0	0	44	84	84
	Kokoniu	26	1-2	0	0	65	88	92
	Arta	15	1-2	0	0	7	100	93
Beyliagan	Orengala	28	0.5-1.3	0	0	89	100	100
	Tiurkler	33	0.5-1.5	0	0	82	94	79
	Khachynabad	32	0.6-1.6	0	0	34	97	84
	Gadekler	30	0.8-1.2	0	0	90	100	100
Belasuar	Fioletovka	32	0.9-4	**8**	**25**	78	97	100
	Tazakend	34	2-4	**10**	**29,4**	91	94	94
	Arkhangelovka	28	1-2	**1**	**4**	71	75	93
Imishli	Jeferly	26	0.5-1.3	**5**	**19**	100	100	96
	YukharyGaragiuvendly	33	0.6-1.8	**3**	**9**	88	97	100
	Khelfaly	34	1-1.9	**2**	**6**	47	85	79
	Sarykhanly	34	0.3-2	**2**	**6**	68	82	76
	Badgyravan	27	0.8-1.9	0	0	85	96	96
Jalilabad	Khalilly	30	1-2	**9**	**30**	100	100	100
	Khajejavadly	33	1-2	**4**	**12,1**	27	48	52
	Laken	29	1-3	**3**	**10,3**	41	100	100
	Khoviuzbiulag	30	1-2	**2**	**6,7**	73	97	83
	Adnaliu	48	0.7-2	**2**	**4,2**	33	85	90
	Biuravar	21	1-2.5	**1**	**4,8**	90	100	95
	Shykhlar	28	1-2	**1**	**3,6**	64	100	89
	Lezran	30	1-2	0	0	47	93	100
Fyziuli	Alhanbeyly	39	1-5	**16**	**41**	90	100	100
	Kerimbeyly	39	1-7	**4**	**10**	67	97	92
Yardimly	Unash	34	0.5-2	**2**	**6**	65	100	100
	Avin	38	0.7-2	**1**	**2,6**	68	97	97
TOTAL / AVERAGE		**939**		**79**	**8.4 %**	**69 %**	**93 %**	**90 %**

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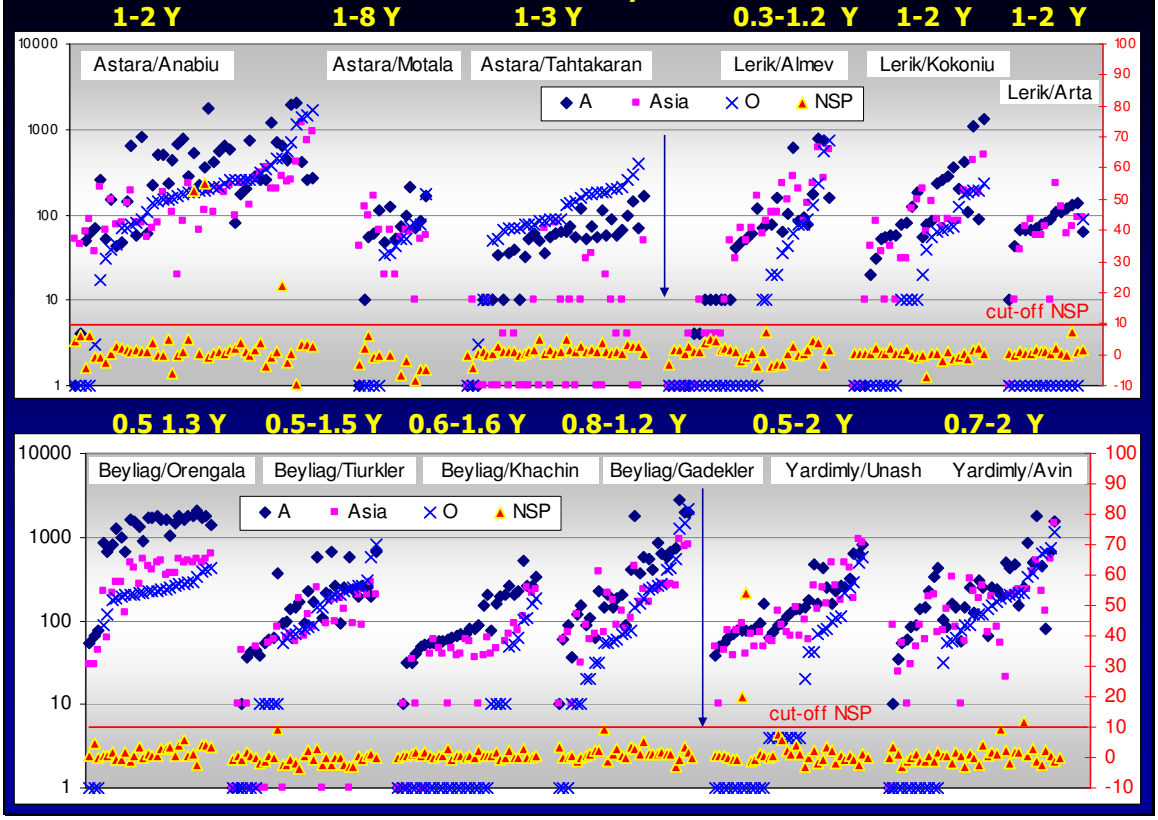
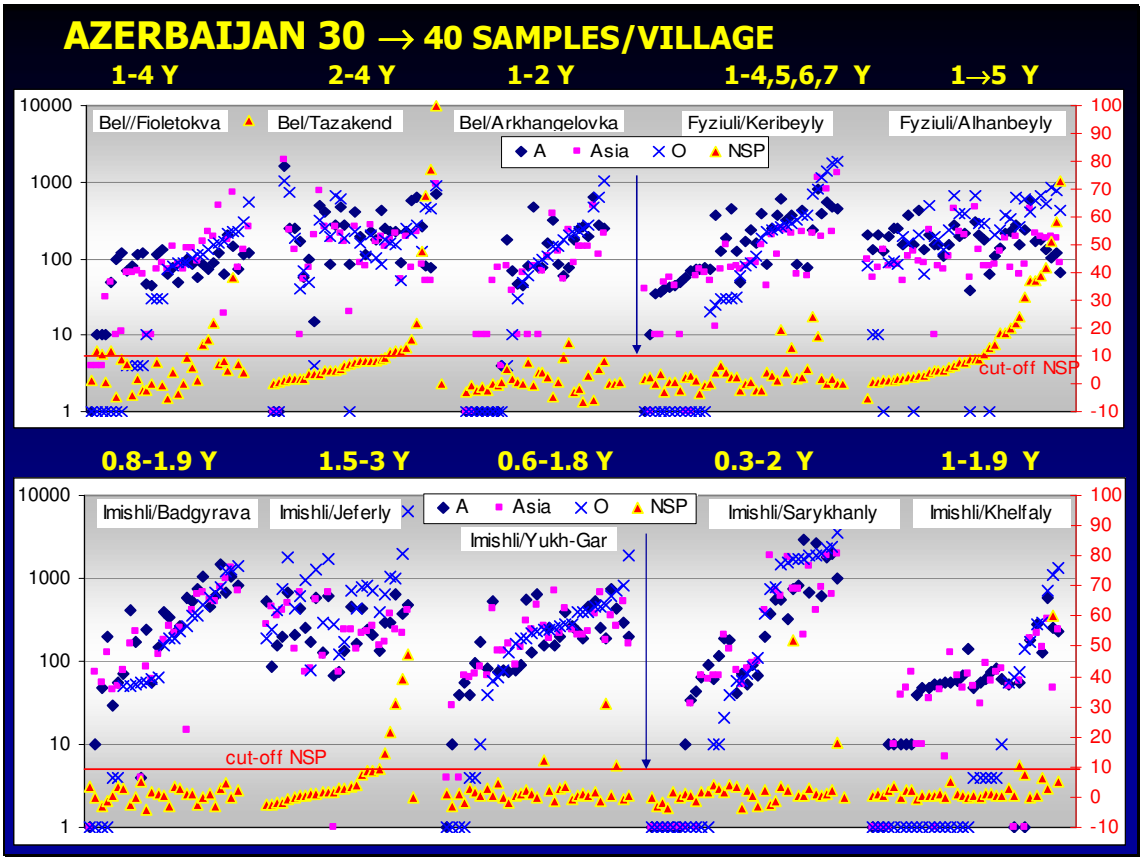
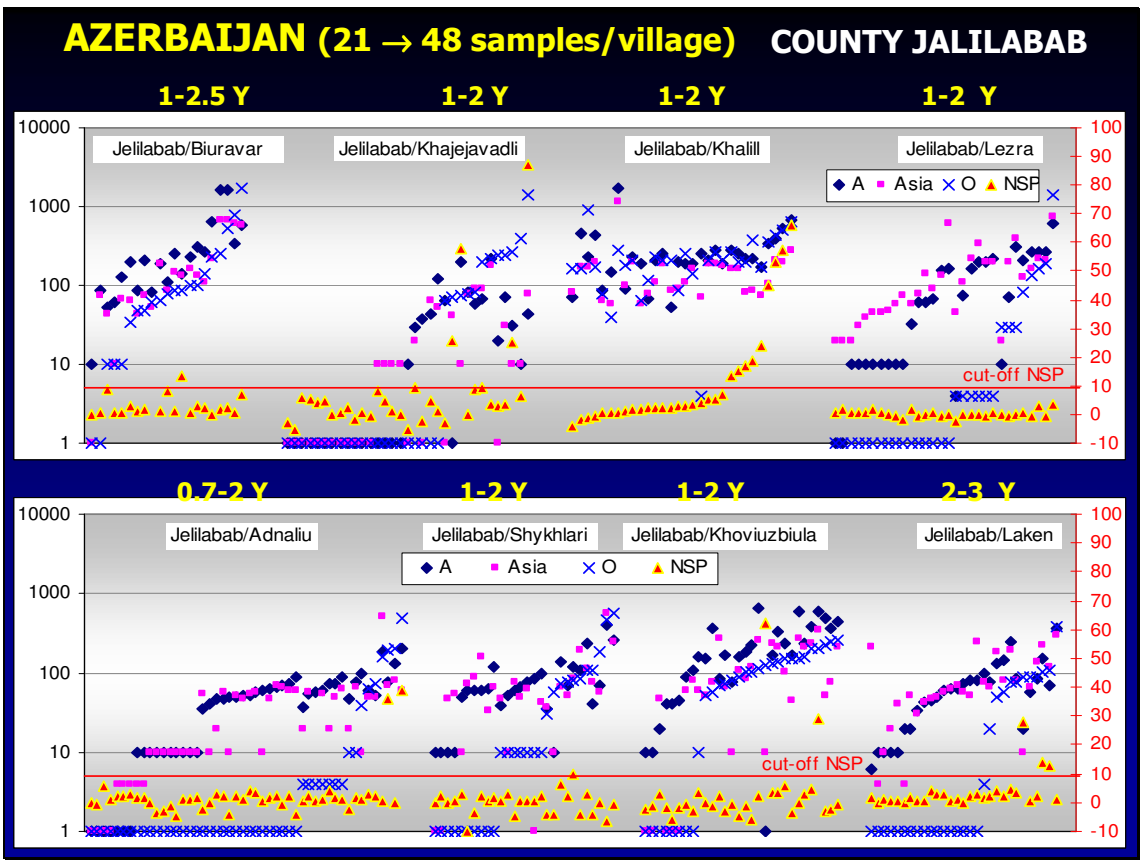
AZERBAIJAN 15 → 40 SAMPLES/VILLAGE




AZERBAIJAN 30 → 40 SAMPLES/VILLAGE
1-4 Y
2-4 Y
1-2 Y
1-4,5,6,7 Y
1→5 Y
Bel//Fioletokva
Bel/Tazakend
Bel/Arkhangelovka
Fyziuli/Keribeyly
Fyziuli/Alhanbeyly
A
Asia
O
NSP
cut-off NSP
0.8-1.9 Y
1.5-3 Y
0.6-1.8 Y
0.3-2 Y
1-1.9 Y
Imishli/Badgyrava
Imishli/Jeferly
Imishli/Yukh-Gar
Imishli/Sarykhanly
Imishli/Khelfaly
cut-off NSP


AZERBAIJAN (21 → 48 samples/village)
COUNTY JALILABAB
1-2.5 Y
1-2 Y
1-2 Y
1-2 Y
Jelilabab/Biuravar
Jelilabab/Khajejavadli
Jelilabab/Khalill
Jelilabab/Lezra
A
Asia
O
NSP
cut-off NSP
0.7-2 Y
1-2 Y
1-2 Y
2-3 Y
Jelilabab/Adnaliu
Jelilabab/Shykhlari
Jelilabab/Khoviuzbiula
Jelilabab/Laken
cut-off NSP

General prevalence of anti-NSP antibodies and vaccinal coverage

Summary	N° cattle	NSP-ELISA Pos	NSP-ELISA % Pos	O Manisa % Pos	A Iran 96 % Pos	Asia 1 % Pos
ARMENIA	900	131	**15 %** (0-93)	**83 %** (50-100)	**92 %** (63-100)	**93 %** (53-100)
GEORGIA	929	23	**2.5 %** (0-33.3)	**47 %** (7-100)	**42 %** (0-100)	**34 %** (0-100)
AZERBAIJAN	939	79	**8.4 %** (0-41)	**69 %** (7-100)	**93 %** (48-100)	**90 %** (51-100)

- ✓ **Virus Neutralisation test** for type O Manisa performed on ≈ 100 sera
  Sera were selected from the three countries and represented various immune conditions (different titres, positive or negative to NSP, ….)
- ✓ Evidence of neutralising antibodies in sera positive by ELISA;
- ✓ Correlation between ELISA and VNT titres;
- ✓ Vaccinal immunity is likely to be protective

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Conclusions

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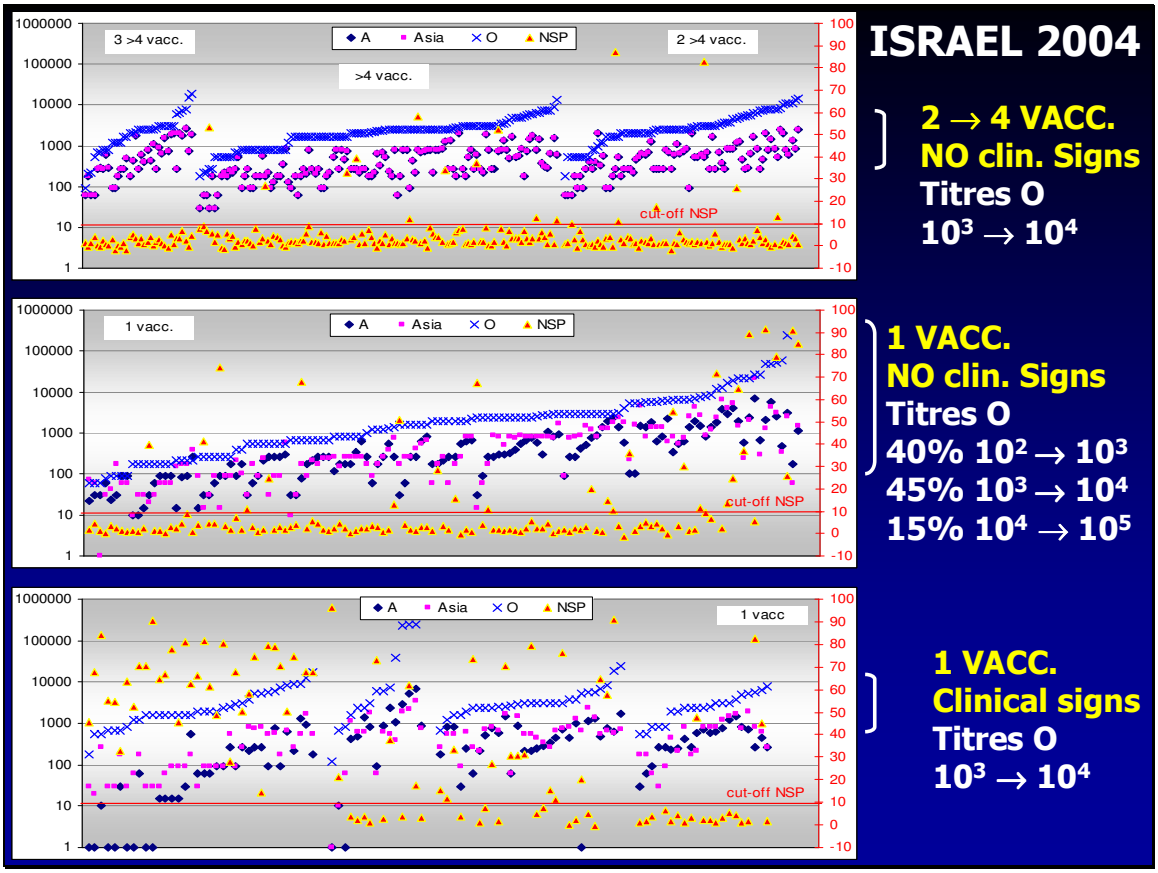


ISRAEL 2004

2 → 4 VACC.
NO clin. Signs
Titres O
$10^3 \rightarrow 10^4$

1 VACC.
NO clin. Signs
Titres O
40% $10^2 \rightarrow 10^3$
45% $10^3 \rightarrow 10^4$
15% $10^4 \rightarrow 10^5$

1 VACC.
Clinical signs
Titres O
$10^3 \rightarrow 10^4$

Appendix 20

ELISA results from Armenia, Georgia and Azerbaijan: statistical evidence for recent FMD circulation?

Matthias Greiner, International EpiLab, Danish Institute for Food and Veterinary

Background

EUFMD RG has requested an analysis of ELISA data collected in May to July 2005 in three selected study regions in Armenia, Georgia and Azerbaijan (see Carsten Pötzsch's presentation of the study for further details) with the main objective to investigate the hypothesis that FMD circulated in the study regions in the last one or two years. This assessment should account for possible positive reactions due to the use of local non-pure vaccine. It can be assumed that older, multiple vaccinates will have more sero-responses than younger (e.g. 1-2 years) animals.

Data

The data sheet was cleaned (age expressed in years for all data from Georgia; The">" sign for high ELISA titres was removed, "<10" was recoded "9", "<=10" was recoded "9.9"; ELISA results labelled with "?" were set to missing; one Asial result "0" recoded "9"; at three spelling versions of "BEYLIAGAN" were unified; region, county (rayon) and location (village or town) were recoded numerically. Three SP ELISAs (Manisa, A Iran, Asia 1) and one NSP ELISA (3abc) were included in the analysis; retest and Cedi results were not considered. For analyses, log-transformed variables were used for Age (logage) and the four tests (spi, sp2, sp3, nsp) (see Tab. 1 for a summary).

Association between age and seroresponse

To our knowledge, some vaccination was used in all study areas. In the absence of more precise information about the vaccination history, we can use the age as a crude proxy for the number of vaccine doses an animal has received during its live. SP reactivity, and to a lesser extent NSP-reactivity due to the use of impure vaccines, should be positively correlated with age under the hypothesis of serological response to vaccination. High anti-SP serum titres in younger animals, on the other hand, could be an indication of recent exposure. Age is an important variable for this analysis. There is some indication for inconsistent age recordings among the three regions (Fig. 1). In Armenia, the age was given in somewhat more crude intervals than in the other regions. The age distribution for Georgia doesn't seem plausible (maybe due to the colour coding to differentiate between month and age recordings in the same variable). A graphical analysis did not reveal any marked association between age and the continuous SP and NSP results for any of the tests or regions (Fig. 2). This is confirmed by linear regression models (outcome log ELISA results versus log age), which did not explain more the 2% of the variation in the serological variable (results not shown).

NSP positivity in relation to SP positivity

While all SP tests are clearly correlated (Fig. 3) the association between NSP and Sp test results is less obvious. It seems that a subgroup of result exist for which higher SP titres are associated with higher NSP titres (Fig. 4). This effect is most obvious for the 0 Manisa and Asia 1 test. A hypothetical explanation could be that some heavily vaccinated animals (i.e. SP strong positive) reacted with the vaccine antigen. It is not possible to rule out an effect of circulating infection in these animals. This analysis used the mean of the SP ELISA results.

The observed number of results for the 16 combinations of the test results was plotted against the expected number, which is generated under the assumption that all test results are independent (Fig. 5). The combination, where all tests except 3abc are positive and the combination of all tests negative occur more frequently than expected.

Distribution of responders

The occurrence of SP and NSP positive test results should be homogeneously distributed over the locations and counties if the assumptions are true that all locations/counties have used the same vaccination regime in the past and no current or past clusters of infection exist.

The distribution of the continuous test results is given for each county in the three regions (Fig. 9-11). Due to the high correlation among the SP tests, the mean of the log transformed results was used for this analysis. In some counties, a marked spread of results into the higher range of the measurement scale is found. This pattern is different among the counties in the three regions and may indicate current or recent FMDV infection or marked differences in the vaccination coverage or efficacy.

Discussion

For Armenia the following vaccination scheme was reported. Armenian bivalent A/O types lapin, 4mg (1995-1998); Russian bivalent sorbent vaccine, 2mg (1999-2002); Russian trivalent A/O/Asia- 1 types vaccine, 3mg (2003-2005); revaccination was made in the given year (Keith Sumption). “Animals are vaccinated in the first year of life, if vaccine is available. They are not booster vaccinated in Georgia and only sometimes in Armenia and Azerbaijan. The vaccination history provided for Armenia does probably reflect the general vaccination policy. It can be assumed that in some years and areas other or no vaccines at all were used. Also, movements and exchange of animals with other areas have occurred” (Carsten Pötzsch). This information does not allow any differentiation of animals according to their vaccination status. More precise data vaccination history (on village level) would allow better interpretation of the data.

The results are consistent with but do not provide a proof of current or recent infection in some counties in the three study regions. Under the strong assumption that the vaccination effect is identical in all study regions, the observed heterogeneity in the SP and NSP positivity rate may be interpreted as an indication of circulating infection in some counties.

As age is a very important variable, the project coordinator may want to check the measurement procedure and outcomes for consistency and plausibility.

Figures and Tables

Table 1: Summary statistics of the data used for the analysis

Variable	Obs	Mean	Std. Dev.	Min	Max
region	2778	2.010799	.8117068	1	3
county	2777	15.19085	8.168278	1	29
village	2777	45.4116	26.4547	1	91
logage	2777	.5139188	.5118739	-2.460214	2.772589
sp1	2761	4.057379	1.682434	2.197225	8.987197
sp2	2762	4.322478	1.606173	1.791759	8.294049
sp3	2761	4.059542	1.449448	2.079442	8.2943
nsp	2763	3.254309	.2580027	1.54	4.934748
omanisa	2761	222.4289	484.3963	9	8000
airan	2762	236.1823	419.7778	6	4000
asia1	2761	161.2585	316.6257	7	4000
abc	2763	3.007928	10.4768	-19.3	115.0381
sp1pos	2761	.6465049	.4781413	0	1
sp2pos	2762	.7483707	.4340279	0	1
sp3pos	2761	.7156827	.4511704	0	1
nsppos	2763	.0839667	.2773881	0	1
age	2777	1.935633	1.300785	.0854167	16
sp	2760	12.43949	4.306758	6.697034	24.3348

Figure 1: Age distribution by region

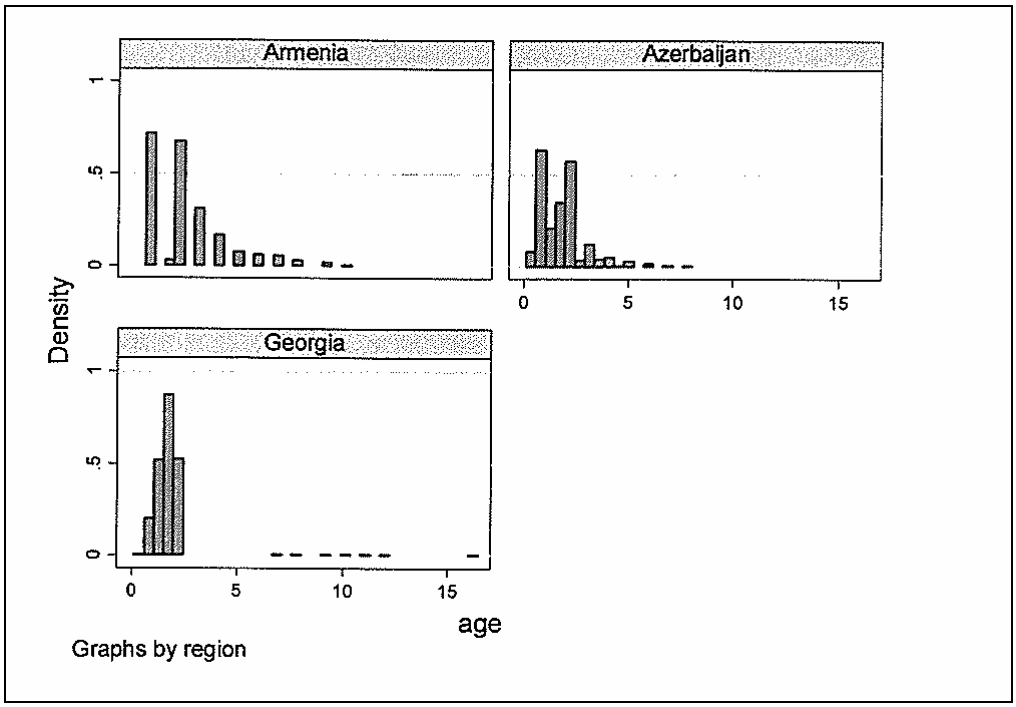


Figure 2: Log ELISA results from top to bottom (O Manisa, A Iran, Asia 1, 3 abc) versus log age.

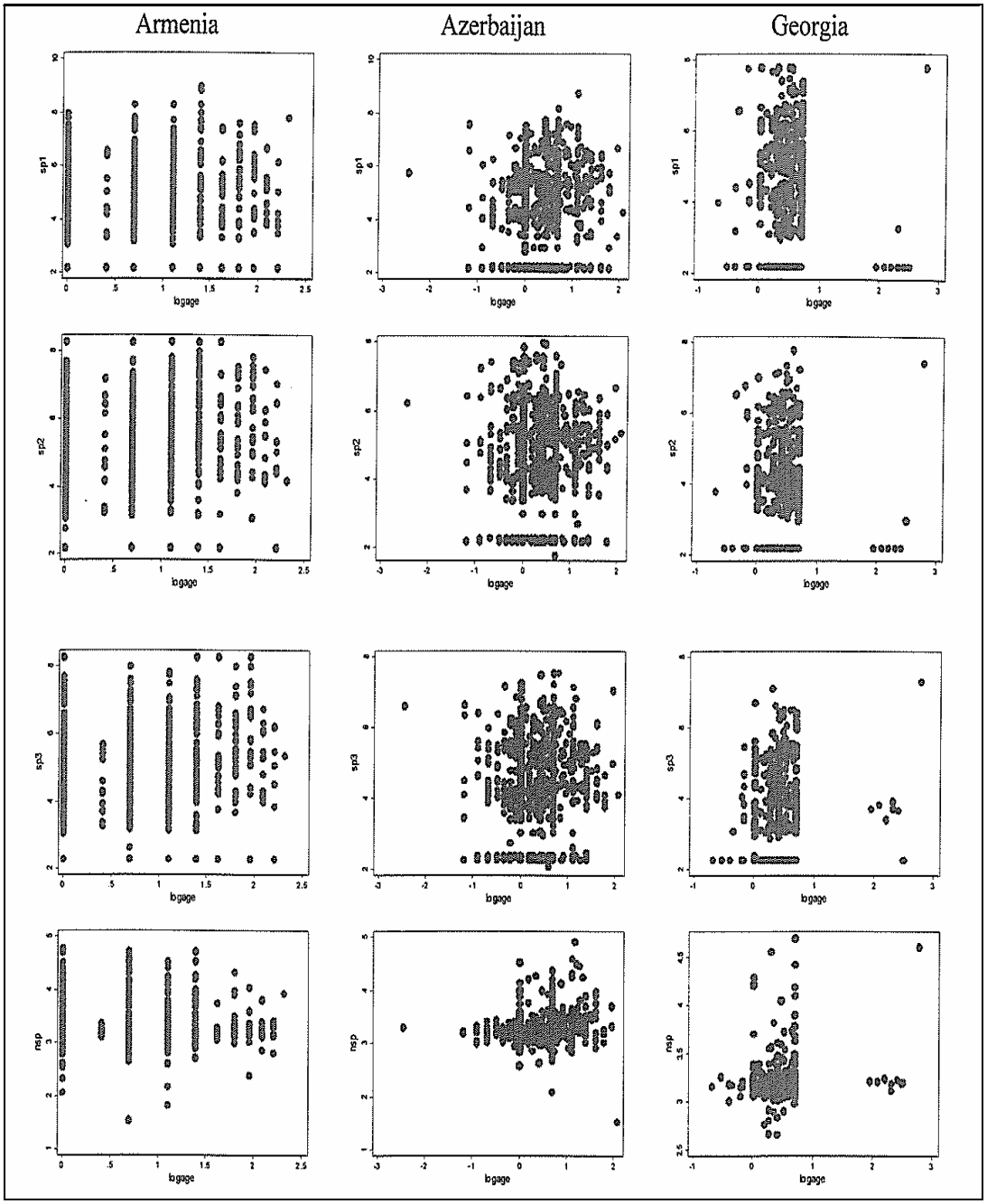


Figure 3: Scatter plots of the three SP tests

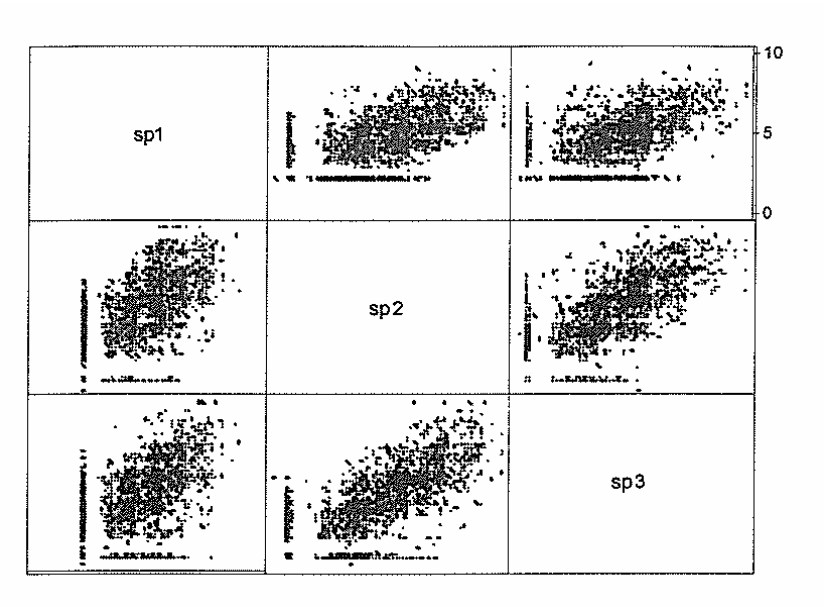


Figure 4: NSP results versus the mean of the results of the three SP tests

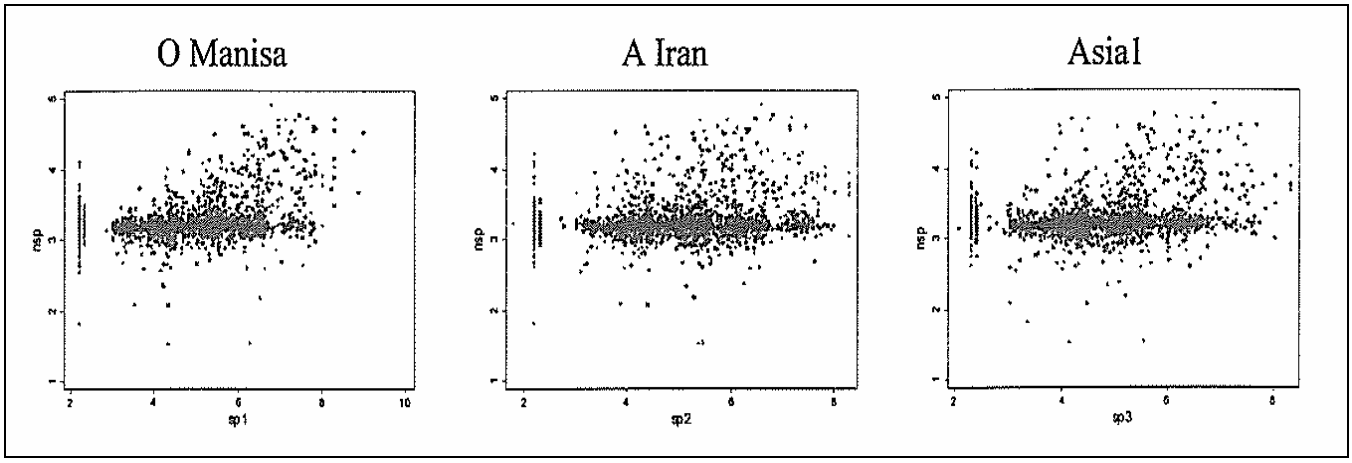


Figure:5 Empirical (obs) and expected (expert opinion under assumption of no circulating infection) probability of positive NSP results in relation to SP test results.

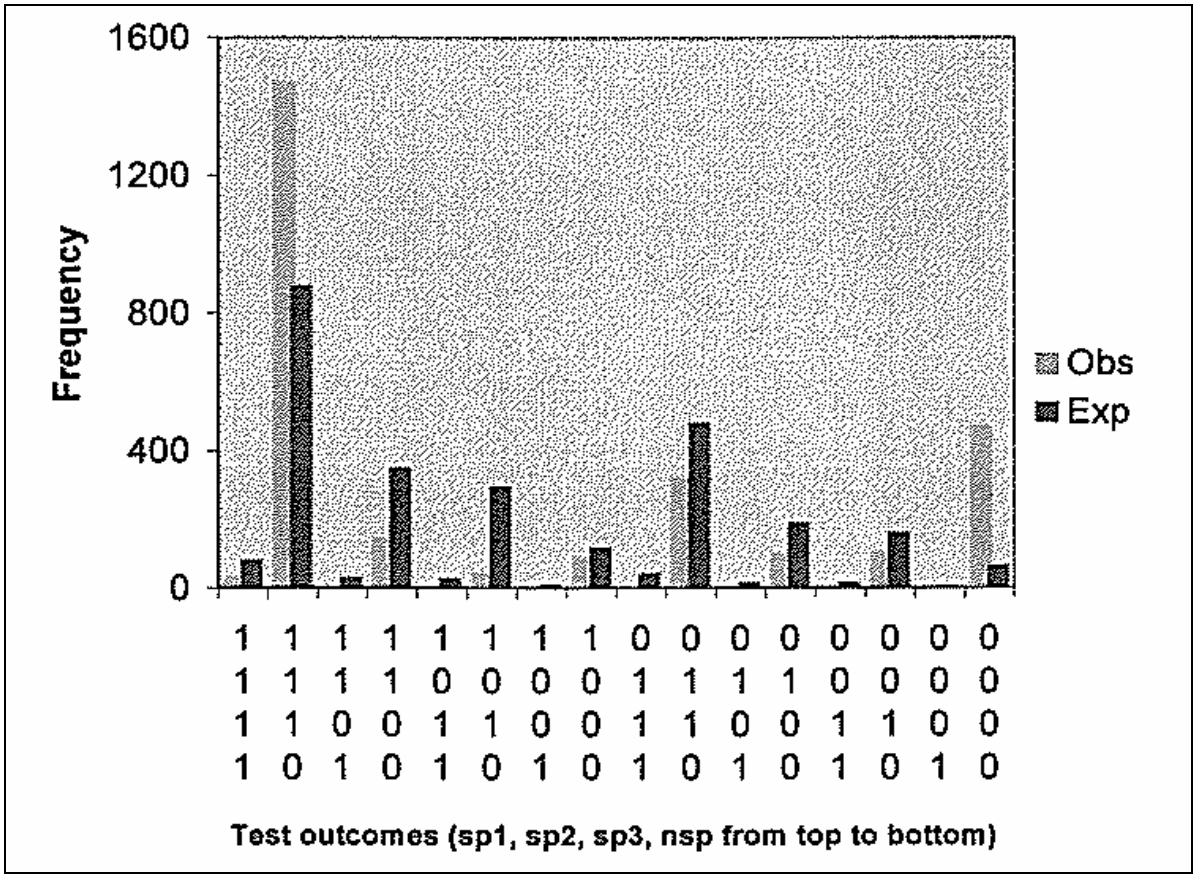


Figure 9: Distribution of (mean) SP and NSP results in different counties in Armenia

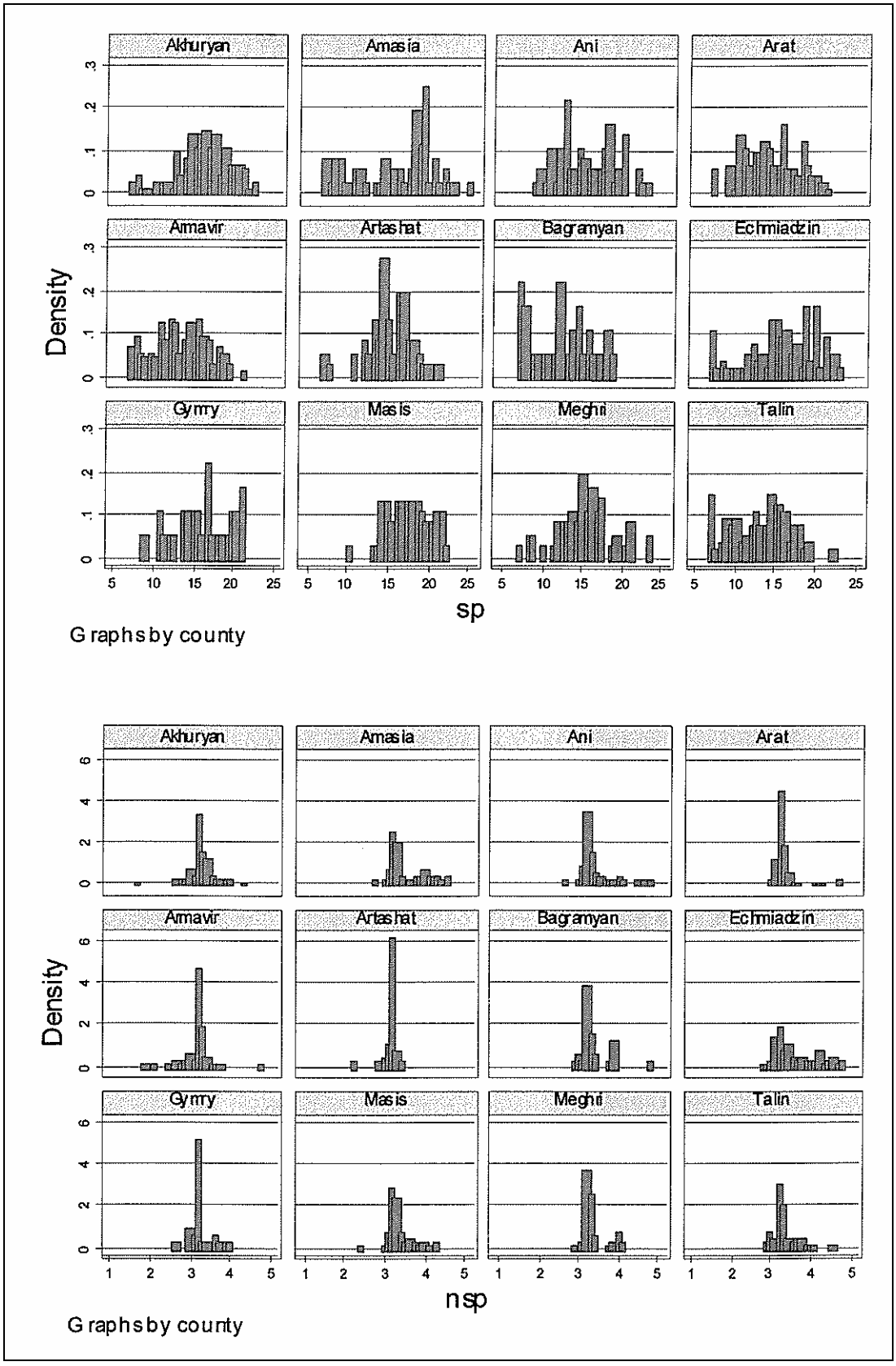


Figure 10: Distribution of (mean) SP and NSP in different counties in Azerbaijan

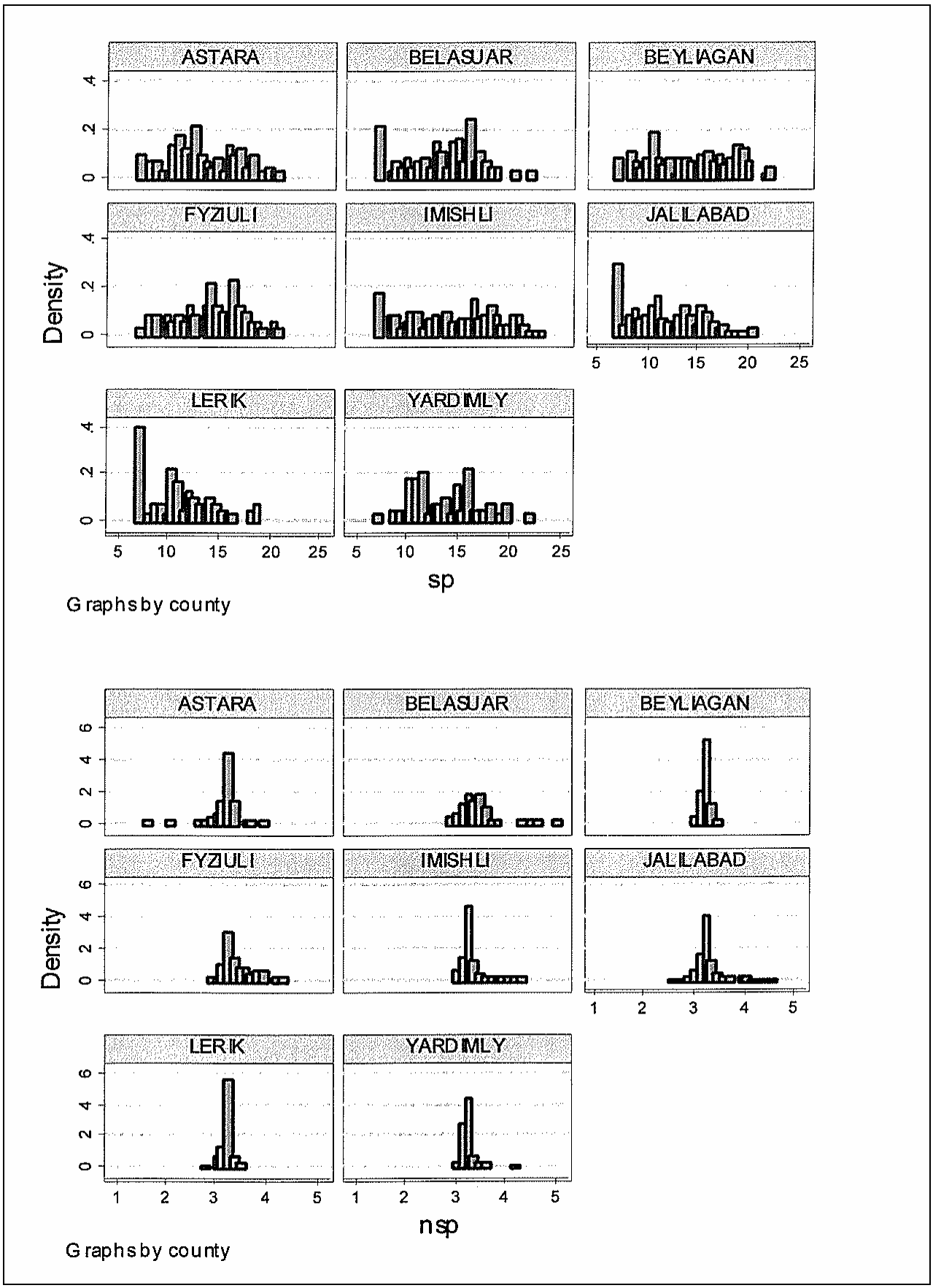
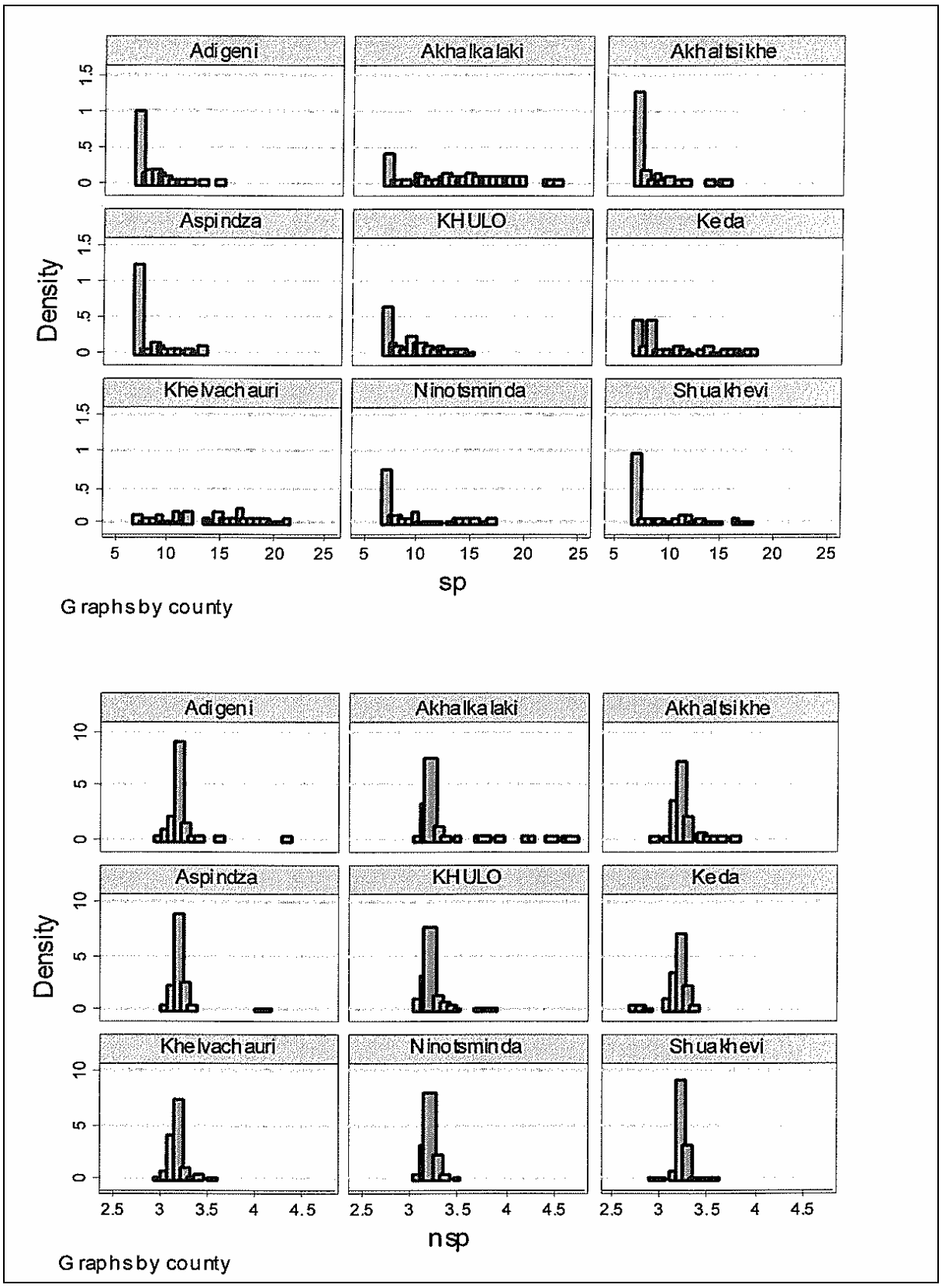


Figure 11: Distribution of (mean) SP and NSP different counties in Georgia.



Outline

- What are decision support systems (DSS)
- What are the decisions
- What are the tools available
- What are the gaps
- What next

2

Why do we need DSS

- Decisions are becoming much more complex
- They often have far reaching effects in other economic sectors
- The livestock industry is undergoing major changes
- Important to make the 'right' decision early
- Non-linearity in effects make prediction difficult and non intuitive - what is right at a local level may not be the best for the national herd

3

Decision Support Systems (DSS)

- Mainly computer systems
- Manage large amounts of data
- Summarise and integrate data to provide information in a timely manner (inferential)
- Allow decision makers to explore various "what if" scenarios (predictive)
- What is the best thing to do (normative)

4

Components

- Database
  - Farms/animals/location
  - Personnel
  - Equipment
  - Transport routes/trade routes
- GIS
  - maps/spatial data
- Models
  - Windspread/Rimpuff
  - Interspread/InterFMD
  - Optimization of resource allocation
  - Prioritising dangerous contacts (DC)

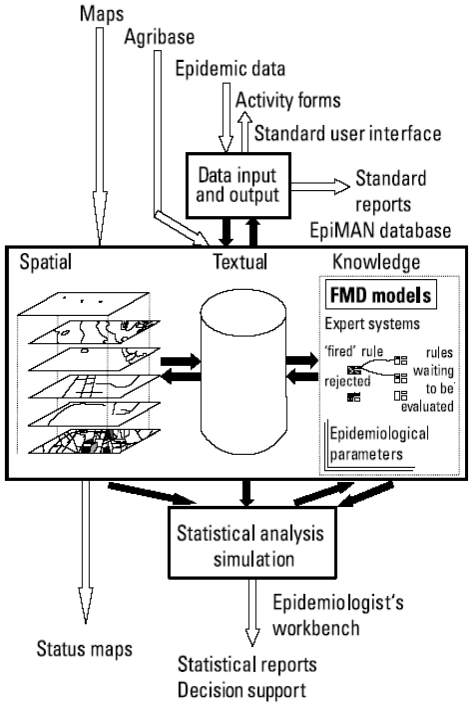


Fig. 5
Schematic diagram of the structure of EpiMAN, showing the various component decision-support tools, and their integration into a complete system

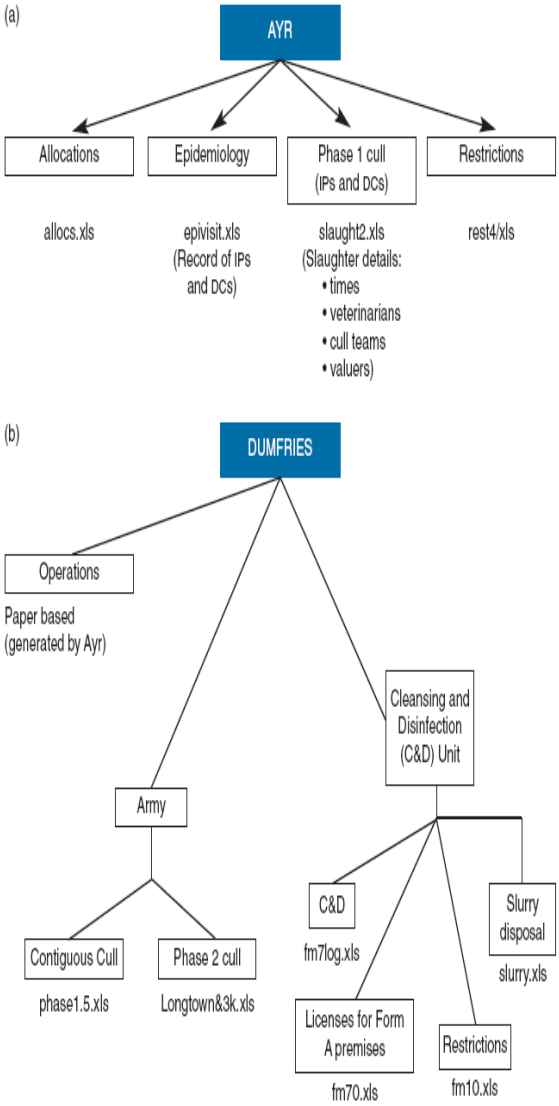
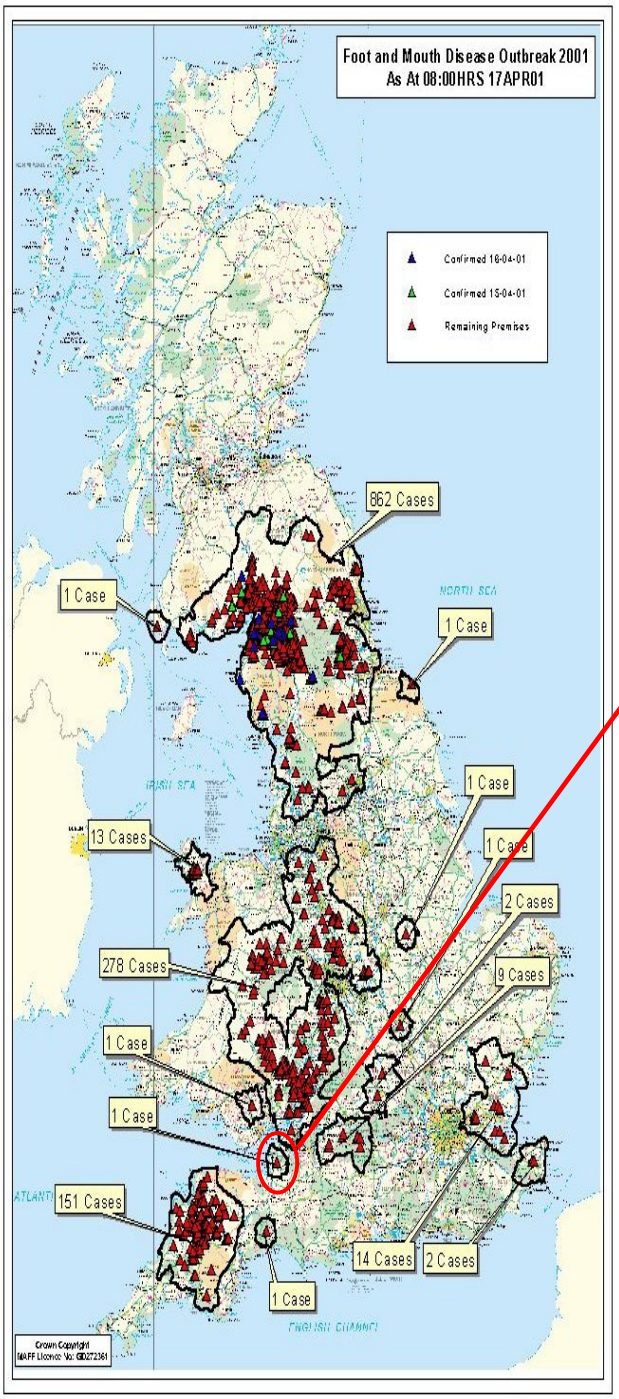


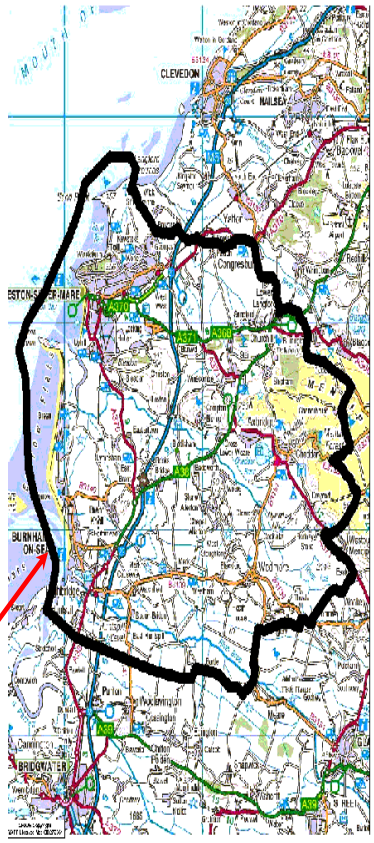
FIG 6: Summary of local databases generated in the control of foot-and-mouth disease in Dumfries and Galloway: (a) Ayr Local Disease Emergency Control Centre, and (b) Dumfries Local Disease Emergency Control Centre. IP Infected Premises, DC Dangerous Contacts

Example of the number of databases being generated during the 2001 outbreak in Dumfries and Galloway

(Thrusfield et al. 2005)

6


Foot and Mouth Disease Outbreak 2001
As At 08:00HRS 17APR01
Confirmed 16-04-01
Confirmed 15-04-01
Remaining Premises
862 Cases
1 Case
1 Case
1 Case
1 Case
13 Cases
2 Cases
278 Cases
9 Cases
1 Case
1 Case
151 Cases
14 Cases
2 Cases
1 Case
NORTH SEA
IRISH SEA
ENGLISH CHANNEL
Crown Copyright


CLEVEDON
BRIDGWATER


ROAD
CLOSED

7

Models

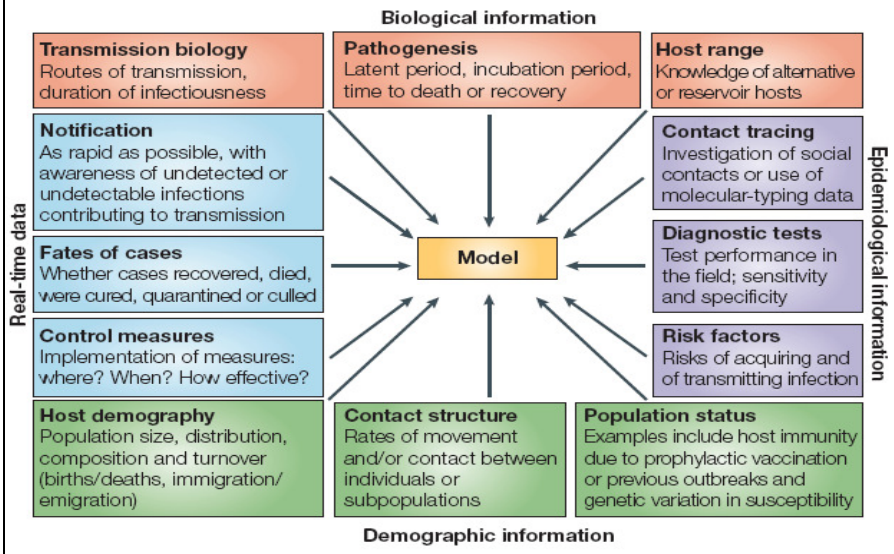
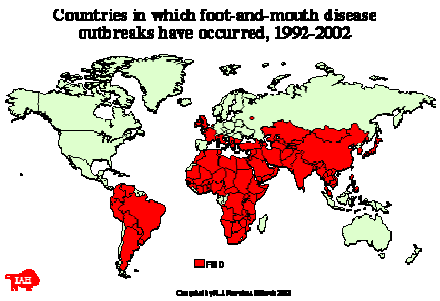


Figure 2 | **From surveillance to modelling.** The schematic shown summarizes the inputs required for construction of a useful model.

(from Matthews and Woolhouse 2005)

8

What are the decisions?



9

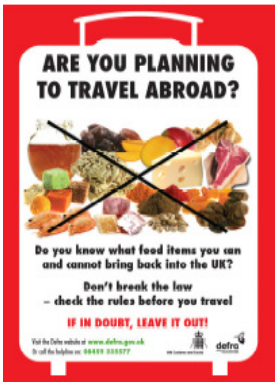
Decisions – control FMD?

- Why control FMD?
- What are the benefits and costs of FMD control in EU?
- How will changes in the CAP affect this decision?
- If we control FMD, do we want to regain
  - 'disease-free' status or
  - 'freedom with vaccination'?
- What can we afford to do?
- If we want to be 'disease-free'
  - stamping out or
  - vaccinate?
- DSS ARE ABLE TO HELP WITH THIS HIGH LEVEL DECISIONS

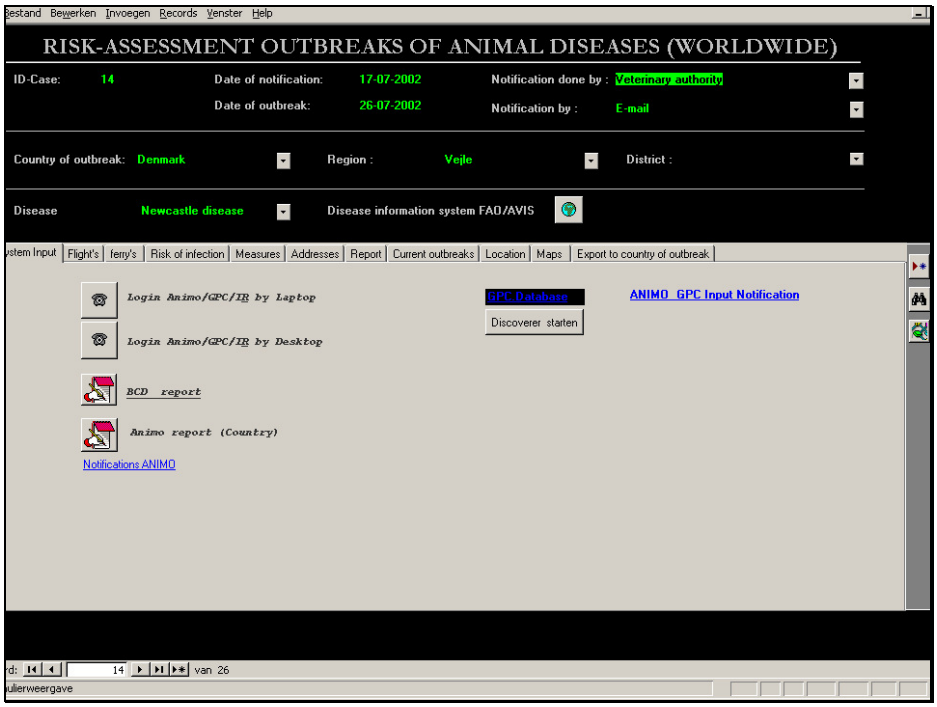
10

Decisions-risk assessments (prevention or eradication?)

- Expanded EU – difficult to control borders
- Once within EU very difficult to trace animal products
- Integrated databases of imports available in real time
- Early warning
- Ready off the shelf assessments for all EU states and for all diseases eg Dutch system

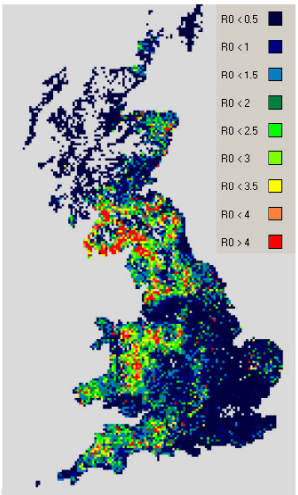


11



Decisions – Outbreak planning

- Where will FMD enter?
- Where to place resources
- Where are the high risk areas
- Where/who are the high risk farms
- Contact networks
  - identify super spreaders



(Source: Risk Solutions 2005)

13

Decisions-start of outbreak

- National movement ban
  - Soon as possible (eg. 2 days earlier in UK 2001 could have reduced outbreak by ~50%)
  - Target markets and dealers to ensure enforcement
  - optimise cost of bans/effect on trade
- Pen-side tests
  - Se/Sp
- Aging lesions
  - Pictures on paper
  - Differentials
  - Digital tech transmit images to a pathologist
- Rapid valuation and slaughter

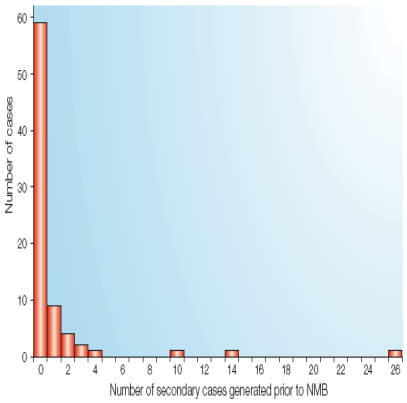
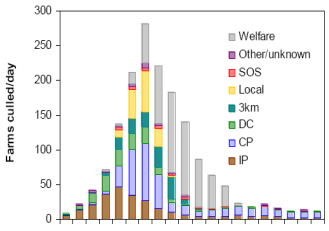


Figure 1 | **Number of secondary cases of foot-and-mouth disease generated prior to the national movement ban (NMB).** The distribution of the number of secondary cases produced by the 78 cases, which were deemed through subsequent outbreak investigation by the Department for Environment, Food and Rural Affairs to have been infected by the time of imposition of the NMB.

Welfare slaughter



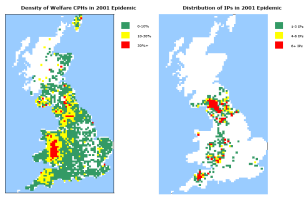


Figure 55: Comparison of distribution of welfare culls with IPs during 2001 epidemic

- What are we trying to optimise?
  - Cost
  - getting back to trade quickly
  - minimise number of slaughtered animals
- DSS WITH ECONOMIC COMPONENTS NEEDED

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Decision – outbreak -airborne spread

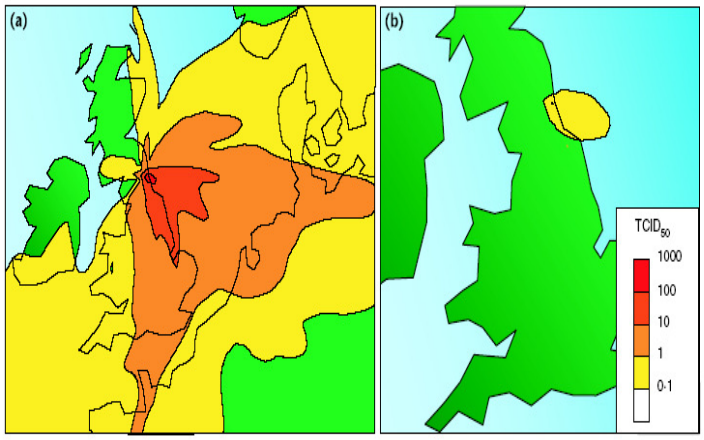


FIG 4: Long-range prediction of the accumulated total cattle inhalation dose in units of $TCID_{50}$ due to the outbreak at Burnside Farm calculated by the DERMA model showing (a) the dose predicted by assuming worst-case conditions and (b) the dose predicted by using the excretion rates which were determined experimentally during the epidemic

Decision-airborne spread

- Several models available
- Dependent on experimental data for reliable estimates
- Newer 'Puff' models include topography
- RIMPUFF (Sorensen 2000)
- Well validated models

- LITTLE EVIDENCE FOR AIRBORNE SPREAD FROM PYRES

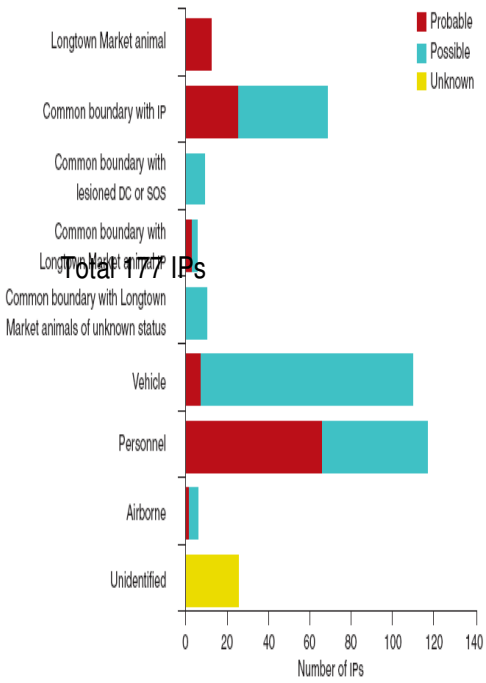
17

Decision –how is epidemic spreading

- What species are affected?
- What parts of the country?
- How many clusters?
- How much long distance spread compared to local spread?
- HOW CAN THIS ALL BE SUMMARISED AND MODELLED TO PREDICT THE EPIDEMIC

How is the disease spreading?

- Long distance
  - animal movement
  - airborne
- Disease spread in 3 km zone
- 70% of UK2001 attributed to local spread
- Number of competing transmission mechanisms
- Poor resolution on local spread

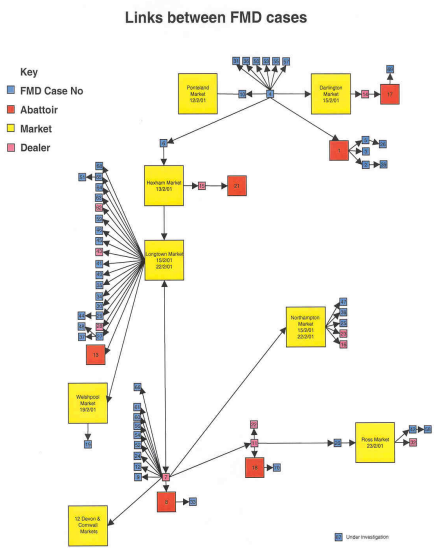


Total 177 IPs

FIG 11: Putative sources of infection of Infected Premises (IPs) in the foot-and-mouth disease epidemic in Dumfries and Galloway in 2001

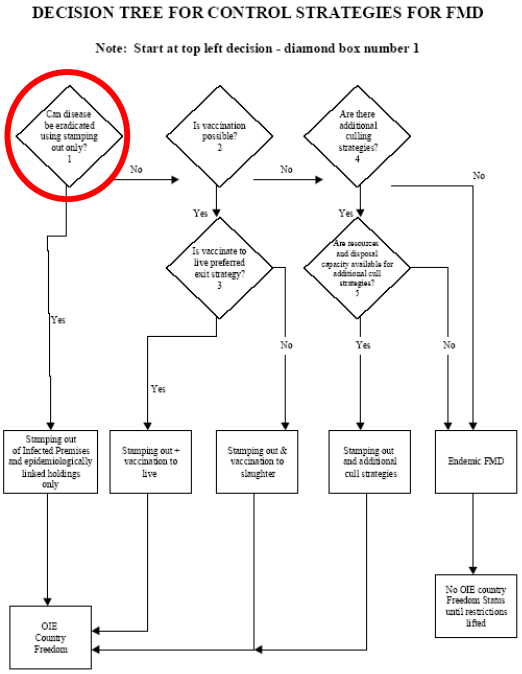
(Thrusfield et al 2005)

Decision - Control methods



- Stamping out
  - IPs only
  - IPs and DCs
  - IPs, DCs and CPs
- Stamping out + vaccination
  - suppressive vacc
  - vacc to live
  - vacc logistic
    - ring
    - regional

20



DEFRA contingency plan 2005

21

Decision - vaccination

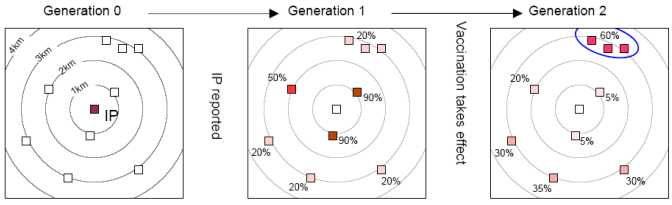
- What serotype and strain is it?
- How many doses are needed?
- Will vaccination work?
- What is the risk of sub-clinical disease/carriers?
- What strategy to use eg. ring, regional, targeted?
- Can the SVS actually achieve the minimum coverage in time?
- Implications for trade etc.

22

Predictive Vaccination (Keeling et al 2003)

Aim:

Use the heterogeneities (both local space and at the farm level) so that vaccination is targeted most effectively.



From identification of the central IP, a secondary model is used to predict those farms currently infected (ie Generation 1). This mode is then iterated forwards to find those farms infected in generation 2 - these can be protected by vaccinating **now**. The model then selects the farms on which vaccination of cattle will have the largest effect.

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Outbreak size	Representative scenario	Control strategy	Mean		95th Percentile	
			IPs	Cost £m	IPs	Cost £m
Small	Norfolk incursion, no windborne virus plumes	IP DC cull only	6	23	15	36
		IP DC cull + 10km vacc cattle	6	38	14	55
		percentage difference	0%	+65%	-7%	+53%
Medium	Cheshire incursion, reference conditions	IP DC cull only	115	116	322	312
		IP DC cull + 10km vacc cattle	85	127	213	284
		percentage difference	-26%	+9%	-34%	-9%
	Cumbria incursion, good DC tracing	IP DC cull only	140	131	299	315
		IP DC cull + 10km vacc cattle	120	159	237	331
		percentage difference	-14%	+21%	-21%	+5%
Large	Powys incursion, high virus infectivity	IP DC cull only	534	437	863	704
		IP DC cull + 10km vacc cattle	248	298	490	529
		percentage difference	-54%	-32%	-43%	-25%

24

Summary from Risk Solutions 2005

- It very slightly reduces the number of infected premises for the smallest outbreaks (only measurable at the 95th percentile level) but has a more significant impact for larger outbreaks (reductions of between approximately 15% and 50%).
- Similarly it reduces the number of animals culled for disease control purposes for larger outbreaks by between approximately 15% and 50%[6]

Other benefits from introducing vaccination include:

- A reduction in the duration of the outbreak if measured from the date of the first reported IP to the date of the last reported IP

However vaccination has some dis-benefits, including:

- A possible slight increase in the number of animals culled for welfare purposes
- An increase in the duration of the outbreak if measured from the date of the first reported IP to the date that official disease free status is regained
- Except in large outbreaks, the vaccination-based strategies are generally more expensive than the IP DC cull only strategy and although the cost differences can be relatively small in absolute terms the additional costs tend to fall more heavily on the livestock industry

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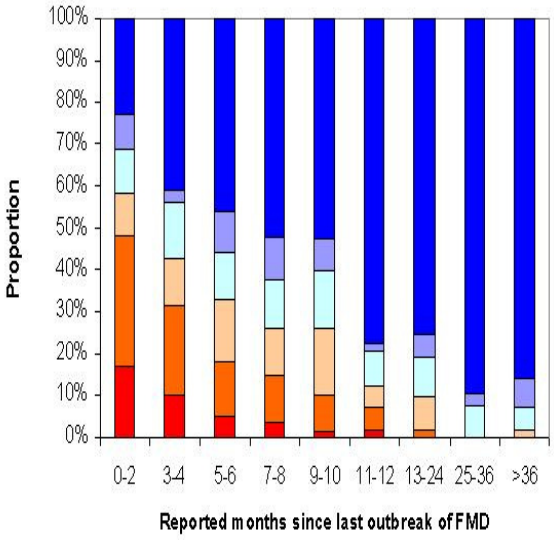
Decision support tool

- Incursion locations
- Virus characteristics
- Regionalisations of economic impacts
- Vet/cull team resources
- DC tracing effectiveness

Key Parameters	Circumstances that favour the "IP DC cull only" strategy	Circumstances that favour the "IP DC cull plus 10km cattle vaccination" strategy
Incursion locations	If the cattle and sheep density in the area surrounding the initial incursion is low to moderate then an IP DC cull only strategy is more likely to be sufficient to manage the outbreak at lowest cost	If the cattle and sheep density in the area surrounding the initial incursion is high then cattle vaccination becomes more cost beneficial
Virus characteristics	A low virus infectivity suggests that an IP DC cull only strategy is sufficient, for example where the virus has a short window of infectivity before clinical signs become apparent and/or does not readily form windborne virus plumes	If the virus exhibits high infectivity characteristics then vaccination is more likely to be cost beneficial, for example where the virus has a long window of infectivity before clinical signs become apparent combined with a tendency to form windborne virus plumes
Regionalisation of economic impacts	If disruption to the meat export trade occurs on a national basis, regardless of the actual size and geographical spread of the outbreak, then a strategy based on vaccination causes extra costs due to the increased time to restore disease free status Consequently the IP DC cull only strategy is more likely to be cost beneficial. If foreign tourists continuing to travel to the UK but simply avoid the areas of the countryside directly affected by the outbreak then an IP DC cull only strategy is more likely to be cost beneficial. If animal welfare problems only occur inside restricted areas (not nationally) then an IP DC cull only strategy is more likely to be cost beneficial.	If disruption to the meat export trade is limited to the regions directly affected by the outbreak only then the large cost associated with the extended time to restore disease free status under a vaccination policy can be substantially reduced. Consequently the vaccination policy is more likely to be cost beneficial. If foreign tourists delay or cancel their visits to the UK as a whole rather than simply avoiding the areas of the countryside directly affected by the outbreak then vaccination is more likely to be cost beneficial because it reduces the duration of the "active" outbreak If animal welfare problems occur on a national basis (i.e. dependent on the duration of the outbreak rather than its size) then vaccination is more likely to be cost beneficial because it reduces the duration of the "active" outbreak
Vet and cull team resource mobilisation (cull delays)	If the 24h / 48h target times for the culling of IPs and DCs can be achieved, especially in early stages of the outbreak, then an IP DC cull only strategy is more likely to be sufficient to manage the outbreak at lowest cost.	If it is not possible to achieve the 24h / 48h target times for the culling of IPs and DCs, especially in early stages of the outbreak, then a vaccination strategy may help to compensate and is therefore more likely to be cost beneficial
DC tracing effectiveness	If the success rate for tracing truly infected dangerous contracts is no worse than was achieved towards the end of the 2001 outbreak then an IP DC cull only strategy is more likely to be sufficient.	If truly infected dangerous contracts are traced less effectively than was achieved towards the end of the 2001 outbreak then a vaccination strategy may help to compensate and is therefore more likely to be cost beneficial.

Key Parameters	Circumstances that favour the "IP DC cull only" strategy	Circumstances that favour the "IP DC cull plus 10km cattle vaccination" strategy
Incursion locations	If the cattle and sheep density in the area surrounding the initial incursion is low to moderate then an IP DC cull only strategy is more likely to be sufficient to manage the outbreak at lowest cost	If the cattle and sheep density in the area surrounding the initial incursion is high then cattle vaccination becomes more cost beneficial
Virus characteristics	A low virus infectivity suggests that an IP DC cull only strategy is sufficient, for example where the virus has a short window of infectivity before clinical signs become apparent and/or does not readily form windborne virus plumes	If the virus exhibits high infectivity characteristics then vaccination is more likely to be cost beneficial, for example where the virus has a long window of infectivity before clinical signs become apparent combined with a tendency to form windborne virus plumes
Regionalisation of economic impacts	If disruption to the meat export trade occurs on a national basis, regardless of the actual size and geographical spread of the outbreak, then a strategy based on vaccination causes extra costs due to the increased time to restore disease free status. Consequently the IP DC cull only strategy is more likely to be cost beneficial. If foreign tourists continuing to travel to the UK but simply avoid the areas of the countryside directly affected by the outbreak then an IP DC cull only strategy is more likely to be cost beneficial. If animal welfare problems only occur inside restricted areas (not nationally) then an IP DC cull only strategy is more likely to be cost beneficial.	If disruption to the meat export trade is limited to the regions directly affected by the outbreak only then the large cost associated with the extended time to restore disease free status under a vaccination policy can be substantially reduced. Consequently the vaccination policy is more likely to be cost beneficial. If foreign tourists delay or cancel their visits to the UK as a whole rather than simply avoiding the areas of the countryside directly affected by the outbreak then vaccination is more likely to be cost beneficial because it reduces the duration of the "active" outbreak If animal welfare problems occur on a national basis (i.e. dependent on the duration of the outbreak rather than its size) then vaccination is more likely to be cost beneficial because it reduces the duration of the "active" outbreak
Vet and cull team resource mobilisation (cull delays)	If the 24h / 48h target times for the culling of IPs and DCs can be achieved, especially in early stages of the outbreak, then an IP DC cull only strategy is more likely to be sufficient to manage the outbreak at lowest cost.	If it is not possible to achieve the 24h / 48h target times for the culling of IPs and DCs, especially in early stages of the outbreak, then a vaccination strategy may help to compensate and is therefore more likely to be cost beneficial
DC tracing effectiveness	If the success rate for tracing truly infected dangerous contracts is no worse than was achieved towards the end of the 2001 outbreak then an IP DC cull only strategy is more likely to be sufficient.	If truly infected dangerous contracts are traced less effectively than was achieved towards the end of the 2001 outbreak then a vaccination strategy may help to compensate and is therefore more likely to be cost beneficial.

Decision - post vaccination sero surveillance

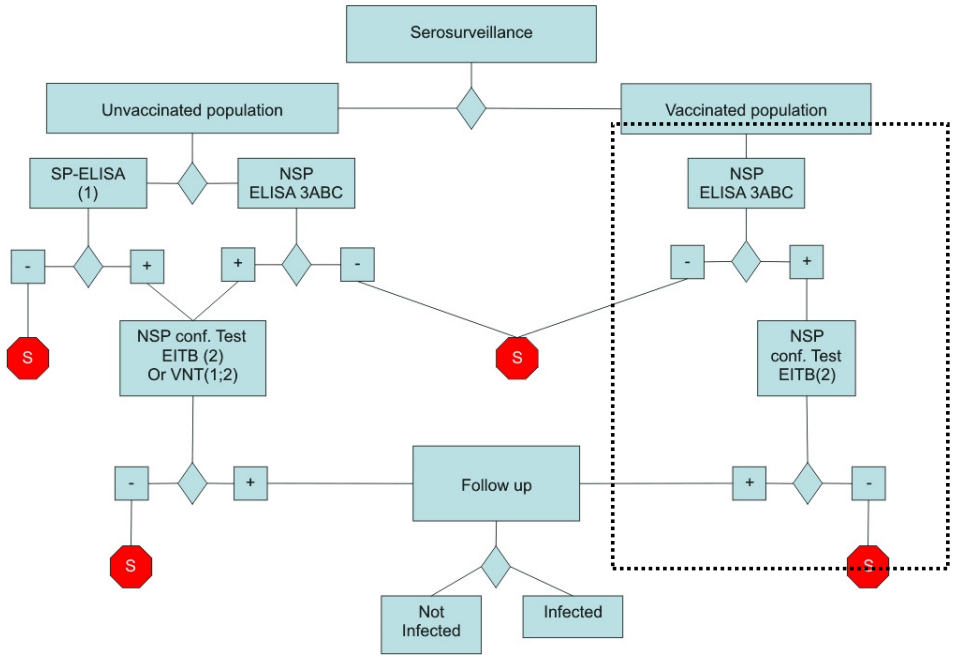


How to do surveillance

- how good is the NSP test
- What is the likely seroprevalence
- Are seropositive herds to be culled
- are carriers identifiable
- serological profiling (Bergmann et al 2000)

27

Two stage testing- OIE 3.8.7



28

Decision - post slaughter clean-up

- What risk do de-populated IPs represent
- How long are they infectious for
- How can they be decontaminated at reasonable cost

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Epidemic models

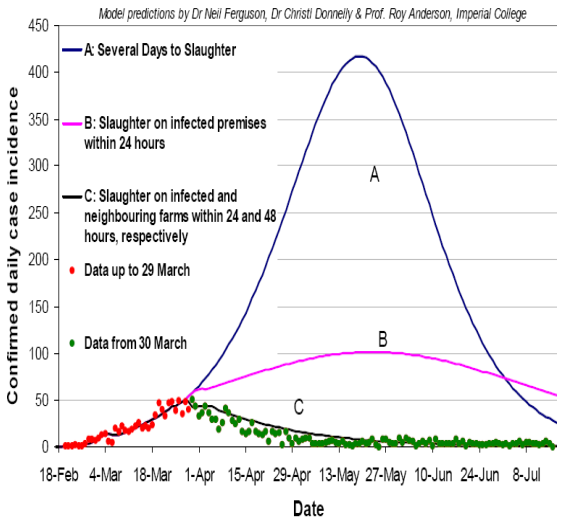
- Imperial model (Ferguson et al. 2001)
- Edinburgh/Cambridge model (Keeling et al. 2001)
- Interspread/InterFMD (Morris et al 2001)
- Silent Spread/ExoDis Model (Risk Solutions 2005)

- Lattice Model (Kao 2003)
- Davis Model (Thurmond et al 2004)

30

Imperial Model (Ferguson et al 2001)

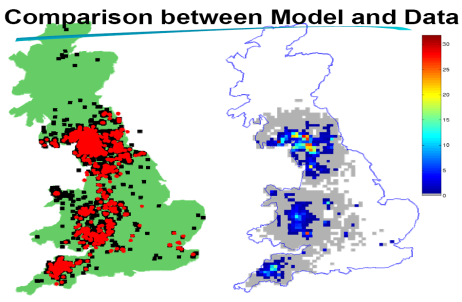
- Differential equations
- few parameters
- quick to run
- assumes random mixing
- not spatially explicit
- deterministic
- no species differentiation



Predictions (as released by OST) made using data up to 29-March.

Edinburgh/Cambridge Model

- Microsimulation model
- few parameters
- computationally intense
- spatially explicit
- stochastic
- does not include airborne spread
- does not include logistics
- vaccination module being added
- models all spread as kernel density function
- accounts for species on farm



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Interspread

- Microsimulation model
- many parameters
- computationally intense
- spatially explicit
- designed as a DSS
  - logistic modules
  - many transmission mechanisms explicit
- airborne spread included
- more widely used
- flexible
- accounts for different species on farm

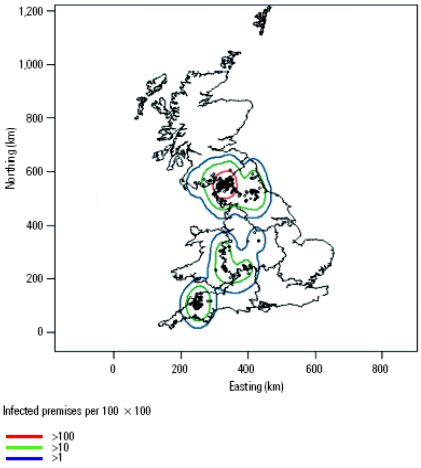

Fig. 4
Contour map of expected distribution of infected farms derived from the InterSpread model, which was used to model national trends almost daily throughout the foot and mouth disease epidemic in the United Kingdom in 2001 (19)

33

Silent spread/Exodis™

Risk Solutions 2005, developed for DEFRA

- Microsimulation model
- uses kernel density function
- also allows explicit modelling of other transmission mechanisms
- airborne module
- logistics module
- vaccination module
- spatially explicit
- moderate number of parameters
- **includes intra-herd dynamics**
- **NEW**

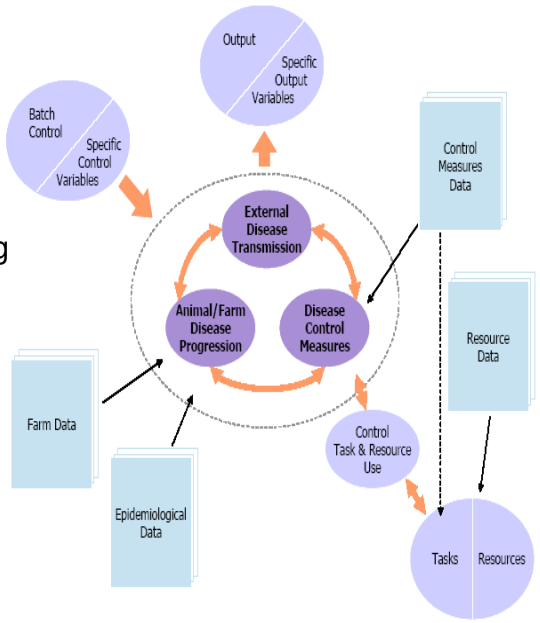

Figure 2: Structure of the Exodis™ framework

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Intra-herd dynamics

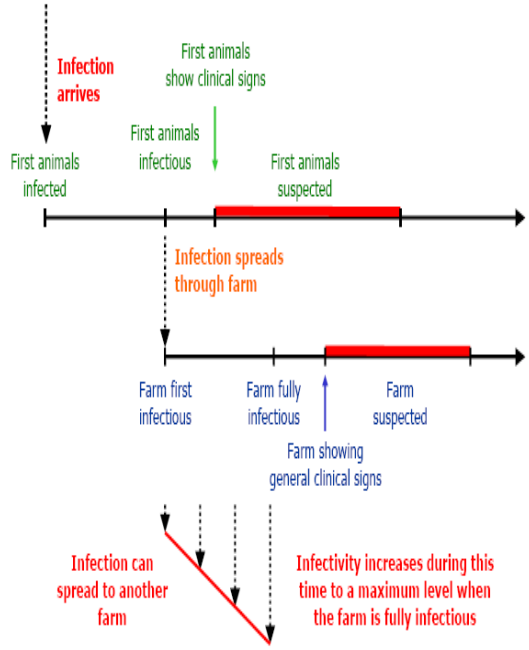

Figure 4: Intra farm dynamics

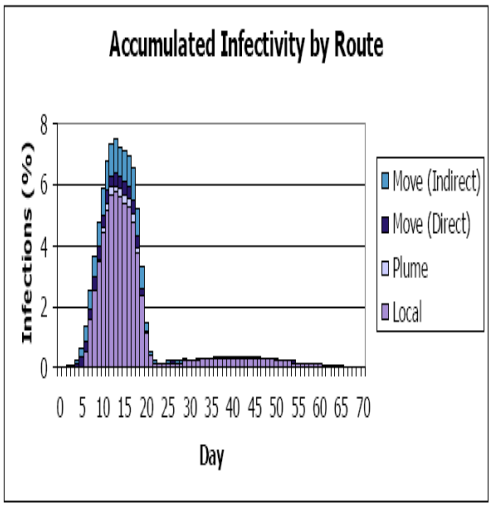

Figure 16: Farm Infectivity by Time

(Source: Risk Solutions)

35

Silent spread/Exodis™

Risk Solutions 2005, developed for DEFRA

- Have combined epidemic model with economic model
- Potentially very powerful

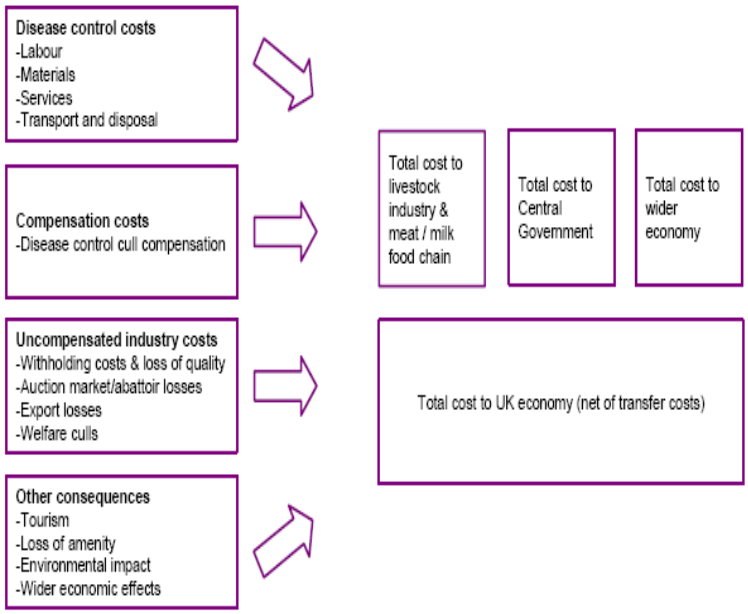

Figure 1: Outline structure of economic consequences models

Comparison of models

	Imperial	Edinburgh/ Cambridge	Interspread	ExoDis
No. parameters	few	few	many	some
Spatially explicit		✓	✓	✓
Different species		✓	✓	✓
Airborne spread			✓	✓
Different transmission mechanisms			✓	✓
Intra-herd transmission dynamics				✓
Logistic/resources			✓	✓
Vaccination strategies		✓	✓	✓

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Models - a word of caution

- All models are WRONG
- They should be used only to SUPPORT decisions
- Very useful for resource planning/allocation
- useful for exploring “what if” scenarios
- They are NOT good at predicting random behaviour
- Models often reflect the biases of the modeller and lack objectivity (James 2005)
- BE CAREFUL OF NUMBERS/ MODELS POSING AS TRUTH (lack

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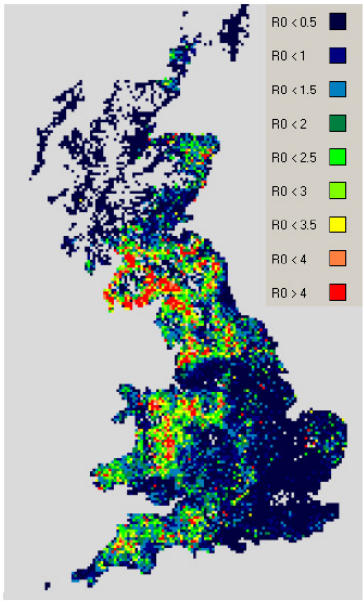
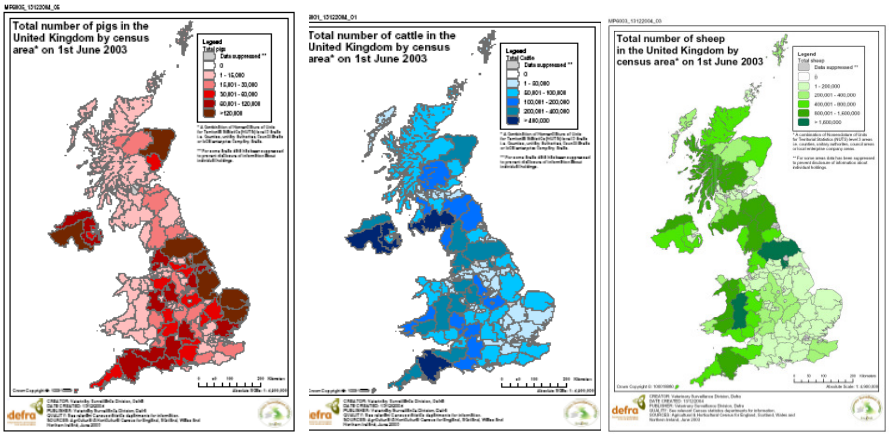
- How good is the model…?



(Source: Risk Solutions 2005)

39



UK livestock densities (Source DEFRA)

40

Actionable items

- Databases
  - trade/contacts/capture
- Improved quality and efficiency of veterinary investigation
- Better understanding of transmission
  - molecular analysis
- Biosecurity
- Economic models incorporated
- Post vaccination surveillance
  - currently underway
- Can we stamp out or vaccinate?
  - Do we have the DSS to call this in time
- Models for endemic FMD control

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GAPS

- Vaccination
- Validation
- Diagnostic test performance
- Epidemiological characteristics of new viruses
- Rapid detection of new cases
- Identifying high risk farms
  - Risk of introduction
  - Risk of spreading
- Local spread

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DSS

- Keep it simple
- Keep it flexible
- Make it transparent

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(Sørensen *et al.* 2000; Keeling *et al.* 2001; Morris *et al.* 2001; Sørensen *et al.* 2001; Kao 2002; Morris *et al.* 2002; Tomassen *et al.* 2002; Kao 2003; Keeling *et al.* 2003)

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Sørensen, J. H., Jensen, C. O., Mikkelsen, T., Mackay, D. K. J. and Donaldson, A. I. (2001). "Modelling the atmospheric dispersion of foot-and-mouth disease virus for emergency preparedness." Physics and Chemistry of the Earth Part B-Hydrology Oceans and Atmosphere **26**(2): 93-97.

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Appendix 22

DRAFT (Version 1)

Minimum standards for bio-security for laboratories situated in FMD infected countries or zones undertaking the testing of samples from holdings with clinical signs indicating the possible presence of FMD

Bernd Haas

The following is a supplement to "Security Standards for FMD laboratories" adapted by the EUFMD General Session in 1993[1].

Introduction

The following Minimum standards for bio-security only appy to laboratories situated in FMD infected countries or zones undertaking the testing of samples from holdings with clinical signs indicating the possible presence of FMD without propagating FMD-virus in cell culture or animals. These laboratories must only employ tests based on reagents where FMD virus infectivity has been inactivated by documented procedures, or those produced by techniques which do not require live FMD virus.

FMD free countries and traditonally FMD free countries attempting to recover the free status after an outbreak should should test samples from holdings with clinical signs indicating the possible presence of FMD only in laboratories meeting the "Security Standards for FMD laboratories" adapted by the EUFMD General Session in 1993. The special buildings and equipment required for such FMD laboratories (more or less equivalent to OIE containment group 4) are expensive and difficult to maintain. In FMD free countries the costs are justified because laboratories capable of employing the full range of diagnostic methods and doing research are an indispensible component of modern disease control whereas any escape of virus from such a laboratory could have catastrophic consequences.

However, in FMD infected countries, the risk of virus escaping from a laboratory without such advanced bio-security features has to be balanced against the advantages of the availability of a diagnostic laboratory in relatively close proximity to the site of outbreaks.

Nevertheless, the risk of virus escaping from a laboratory has to be mitigated by appropriate provisions. As long as the virus is not amplified in cell culture of infected animals, the risk of FMD occurring as a result of diagnostic activities within laboratories is associated with escape of virus following receipt of samples from infected animals. Any generation of infectious aerosols should be limited as far as possible and preferably be restriced to a laminar flow cabinet. Efluents possibly containing FMD infectivity should be collected and treated.

Minimum Requirements

Personnel

1. A disease security officer (DSO) and deputy (DDSO) must be designated, and one or both present on-site at all periods in which samples are being received and contactable at all periods when sero-diagnostic activities are ongoing.
2. The DSO/DDSO must have sufficient experience and technical training to enable assessment of FMD risk and risk management procedures.
3. There must be a designated restricted area or areas with controls in place to limit human access
4. Personnel must be authorised to enter the restricted area by the DSO/DDSO.
5. Authorised personnel working in the restricted area must be trained in disease security and evidence of the training recorded. Where facilities for the inactivation of waste from the restricted area are located outside of this area, also staff working with such waste must be trained in disease security and evidence of the training recorded.
6. ***Authorised personnel must change clothing before entering the restricted area and take a shower when leaving the restricted area. If possible, authorised personnel should not have any contact to animals of susceptible species, enter buildings or enclosed fields where animals of susceptible species are kept, and handle items used in the care of susceptible species, for at least 3 days after leaving the restricted area.***
7. Entry and exit of personnel to the restricted area should be recorded.
8. Entry and exit points to the restricted area will be kept to the minimum – preferably a single point of entry/exit.

[1] Appendix 6 (ii), Report of the 30th Session of the European Commission for the Control of Foot-and-Mouth Disease, Rome, Italy, 27-30th April, 1993.

9. A step-over line, or other clearly demarcated boundary shall indicate the exit point.
10. In case the shower facilities are not placed at the border of the restricted area, outer protective garments, including shoes or shoes coverings, shall be removed before exit from the restricted area. All clothing worn in the restricted area must be stored in a secure way, e.g. in designated lockers, until treatment.
11. An incident recording system must be in place to ensure early notification of the authorities responsible for FMD surveillance in the event that samples have been received in unsatisfactory state of packaging.

Buildings

12. Susceptible livestock must not be kept on the premise where the restricted area is located.
13. Access doors to the restricted area should display a warning sign that access is restricted to authorised personnel only.
14. Changing facilities and lockers are required to enable staff to deposit unessential items outside the restricted area.
15. Entering of the laboratory premises by farmers or staff working on farms should be avoided. If possible, it should be attempted to separate vehicles bringing samples from vehicles entering the premise for other purposes.
16. Shower facilities must be available onsite, preferably at the border of the restricted area.
18. Sample reception area
    a. The restricted area must contain a specified area for reception of packages.
    b. This area must:
        i. Be easily disinfectable in the event that leakage of samples occurs into packing materials or following opening of the packages;
        ii. Have suitable facilities for waste disposal and have hand-washing facilities at exit points.

19. N.A.

20. Sample testing area
    a. The restricted area must contain a specified area for testing
    b. This area must have suitable facilities for surface disinfection and waste disposal and have hand-washing facilities at exit points

21. N.A.

22. Communications and reporting office space
    a. The laboratory housing the FMD serology facility must demonstrate an adequate provision of office space, computing and communications facilities and organisation, sufficient to reduce the need to a minimum for staff, papers and physical records to exit the restricted area on a daily basis.
    b. Facilities should be in place (for example, electronic communications, facsimile) to prevent any need for untreated papers to exit the restricted area.
23. Rest rooms
    a. The restricted area should have sufficient rest rooms and lavatory facilities in relation to the staff number expected at peak periods of activity.
    b. The laboratory housing the FMD serology facility must demonstrate adequate provision of such facilities, sufficient to reduce the need to a minimum for staff to exit the restricted area on a daily basis.
24. Location of autoclave
    Facilities for wet heat treatment must be present on the site, preferably with sufficient capacity for throughput at the maximum operating capacity of the serology laboratory. (See also 26. Solid waste)

Waste

25. Liquid waste
    a. Heat or chemical treatment of all waste water is the PREFERRED treatment, in compliance with the prescribed standards specified for FMD laboratories.
    b. Alternatively, or additionally, the laboratory may demonstrate that it has put in place a system for inactivation of virus if present in liquid waste that has contacted risk materials. If treatment of all liquid waste from the restricted area (including waste water from the showers) is not possible, at least the ELISA buffers and washing fluids must be collected and treated.
26. Solid waste
    a. For biological, solid waste, and all solid disposable materials that have been in contact with specimens, treatment by wet-heat, in accordance with the level of heat effect specified for security standards for FMD laboratories, in an autoclave within or at an entrance point to the restricted area is the preferred option.
    b. If such a treatment of all solid waste is not possible, it may be packed into suitable watertight containers and, after spraying of the containers with disinfectant, removed for treatment at a different site.

27. Removal of equipment, materials and clothing from the restricted area

a. Removal of any equipment from the restricted area shall be subject to authorisation by the disease security officer.
b. The reason for removal, date, and destination will be recorded, precautions taken to reduce potential virus contamination.
c. The DSO will ensure that equipment which has been in contact with risk materials (specimens) will not be removed from the restricted area without treatment in accordance with the procedures given in the Security Standards of FMD Laboratories.

28. Declassification of the restricted area
    a. A decontamination plan must be agreed with the competent authorities, before restrictions can be lifted.
    b. If heat treatment or scanning of all paper from the restricted area is not possible, it should be packed into suitable containers, which should be disinfected and kept under lock for at least two years. If the containers have to be opened before, this has to be done in a restricted area meeting the standards described above.

Packaging of samples for holdings with clinical signs indicating the possible presence of FMD
Samples must be put into watertight primary containers (e.g. plastic tubes) and the primary containers must be packed in watertight secondary packaging, which should be a strong crushproof and leak-proof container, with absorbent material that can absorb the entire contents of all the primary containers. The packaging process must include a disinfection of the secondary packaging. Preferably, the packaging should comply with the European agreement concerning the international carriage of dangerous goods by road (ADR).

		OIE containment group		
		2	3	4
FMD- Status	Free without Vaccination			*
	Free with vaccination			*
	"Trying to recover free status"	**2 + for serology**		*
	FMD infected	**2 + for diagnosis without amplification**		

* Existing facilities built after FAO FMD-lab specifications may not fully meet current OIE containment group 4 requirements (No HEPA filters for input air, but gas tight hatches; double HEPA filters only for stables). However, this is not considered a significant disadvantage and should be tolerated at least until such facilities undergo a major renovation.

Appendix 23

Virus Inactivation Kinetics

Soren Alexandersen
Danish Institute for Food and Veterinary Research, Department of Virology, Lindholm, DK-4771 Kalvehave, Denmark

At the session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease in Chania, Crete, Greece, FMDV inactivation kinetics were again discussed and it was concluded that more experimental data was needed but that existing data should be thoroughly reviewed and be the basis for subsequent specific recommendations for experimental studies to be performed. It was agreed that Prof. Matthias Greiner would facilitate a review of the available data on FMDV inactivation in meat and meat products and that Prof. Soren Alexandersen would facilitate a review of existing data on FMDV inactivation in milk and milk products. The findings and conclusions in regard to meat and meat products will be presented separately by Matthias Greiner. The existing knowledge in regard to FMDV excretion, transmission and stability of FMDV relevant for estimating the risk of raw and treated milk and milk products is briefly reviewed below and has been previously reviewed by Donaldson and by Haas (Donaldson, 1997; Haas, 2003). The review below focuses in particular on areas of uncertainty where suitable data are sparse or missing.

Virus and target and host range. Foot-and-mouth disease virus (FMDV) is classified within the *Aphthovirus* genus in the *Picornaviridae* family (Bachrach *et al.*, 1957; Newman *et at*., 1973; Belsham, 1993; King *et al.*, 2000) and is the causative virus of foot-and-mouth disease (FMD), a vesicular disease which with some minor exceptions affects members of the order *Artiodactyla*, i.e. all cloven-hoofed animals including domestic and wild ruminants and pigs (Thomsen, 1994; Thomson *et al.*, 2003; Alexandersen & Mowat, 2005). The animals which under natural conditions are of greatest significance include cattle, pigs and small ruminants (sheep and goats) and, in particular in Asia and South America, the water buffalo. African buffalo play an important role as the natural maintenance host in Africa, but other wildlife such as impala may also be involved in the natural epidemiology of FMD. Animals which may contribute to the transmission of virus under certain conditions or which cannot be excluded as having some risk of transmission include deer, camels, llamas and alpacas, any animal of the order Artiodactyla and Indian elephants. These animals may not easily become infected but can potentially be of some significance if they get in close contact with livestock, for example when kept under farmed conditions or in zoos (Alexandersen & Mowat, 2005). However, whether such animals, e.g. camels, could play any role in transmission of FMDV by milk and milk products are currently unknown.

FMDV is highly variable with seven serotypes identified and it cannot be excluded that heat stability may vary among serotypes or subtypes.

Transmission and prevalence. FMD virus can be transmitted by many routes, including airborne spread, and may in addition to the usual acute vesicular disease cause a subclinical, persistent infection in ruminants (so-called "carrier" animals) (Van Bekkum *et al.*, 1959a; Van Bekkum *et al.*, 1959b; Sutmoller & Gaggero, 1965; Alexandersen *et al.*, 2002; Alexandersen *et al.*, 2003b). FMD spreads very efficiently when first introduced on a farm, and the prevalence on any given farm is likely to be rather high before disease is noted, in particular if there is free contact among animals. Furthermore, dairy cattle are the animal group having the highest relative risk of becoming infected according to common experience and also recently shown by quantitative modelling studies of epidemiological data (Keeling *et al.*, 2001; Ferguson *et al.*, 2001a; Ferguson *et al.*, 2001b; Keeling *et al.*, 2003). Nevertheless, as the clinical signs of FMD in cattle, in particular in dairy cattle, are usually obvious, it is considered unlikely that more than a few dairy herds may become infected before suspected FMD is reported to the official veterinarian. Consequently, for the purpose of analysis one may assume that only a few dairy herds are affected at the time of reporting, but that the prevalence of FMD may vary

from 10-90% among tile dairy cattle in an individual herd. As disease progresses and lesions may develop on the udders, affected animals may become difficult to milk and milk production may drop dramatically. Furthermore, such animals are more likely to develop mastitis, affecting the quality and quantity of milk produced from infected farms. However, the risk of spread of FMDV by milk is mainly in the early phases of the infection before disease is officially reported and procedures put in place. Therefore, one may assume that the production of milk from fanns affected early on will be at normal levels and of normal quality.

Excretion. FMDV is shed to all secretions and excretions, including semen, during the acute phase of the infection. FMDV can replicate in the squamous epithelia of the mammary glands of dairy cattle (Burrows, 1968; Sellers *et al.*, 1968; Sellers *et al.* 1969; Burrows *et al.*, 1971; Burrows *et al.*, 1981; Blackwell *et al.*, 1981; Blackwell & Yilma, 1981; Blackwell *et al.*, 1982) and virus is consequently excreted in significant titres in milk from shortly, i.e. up to 2-4 days, before clinical signs appear and through the 4-5 days of the clinical phase, in a pattern that largely mirrors the viraemia profile. After the high level excretion during the acute phase, very low levels of FMDV have been reported to be excreted in milk for up to 3 weeks or more (Burrows *et al.*, 1971). Not much is known about the content of FMDV in milk from other species than cattle, but a positive reaction (RT-PCR) for FMDV in the milk from experimentally infected sheep has been reported (Callens *et al.*, 1998) and work by McVicar and Sutmoller (McVicar & Sutmoller, 1971) showed high levels of virus in the milk from infected goats. Thus, one should assume that the milk from other species than cattle also contain relatively high titres of FMDV. Large amounts of virus are excreted in vesicular fluid, in desquamated vesicular epithelium and, in cattle, also in saliva (Hyslop, 1965; Scott *et al.*, 1966; Cottral, 1969). There is also excretion, but to a much lesser extent, in faeces and urine (Burrows, 1968; Parker, 197!; Garland, 1974), in a pattern that also reflects the peak of viraemia, lesions and clinical disease. A sharp decline in viral excretion and load occurs around day 4-5 of clinical disease, when a significant circulating antibody response is detectable.

In regard to levels of FMDV shed in milk, Donaldson et al 1982 (Donaldson *et al.*, 1982) mention individual milk titres of $10^{0.7}$-$10^{6.6}$ TCID50/ml in samples collected from six clinically normal cattle during the 1981 Isle of Wight outbreak and in a single bulk tank milk (the herd had 32 milking cows) of $10^{2.2}$ TCID50/ml. Thus, we may assume an average FMDV titre of 10^2 TCID50/ml milk from infected farms and we may also assume that the bulk tank milk from a single infected herd go through a further dilution of 10-fold into uninfected farms milk (into a collecting tank), then the average titre would be 10^1 TCID/ml milk in the collecting tank (bulk). However, it is not unlikely that the titre in certain situations may be much higher, e.g. titres of $10^{5.5}$ TCID/ml and 10^4 mouse infectious doses (corresponding to approximately 10^5 TCID50) per ml have been found in a milk chum and in both a milk tanker and a retail bottle of milk (Anonymous, 1969; Hedger & Dawson, 1970; Burrows *et al.*, 1971).

Interference from vaccination. Shedding of FMDV in the milk from vaccinated animals is likely to be at a lower level than from non-vaccinated animals (de Leeuw *et al.*, 1978; de Leeuw, 1980). However, as vaccination against FMD in the EU are expected to be reactive, i.e. to potentially be started after the first outbreaks are reported, the risk of excretion of FMDV in the milk of infected ruminants before disease reporting is not affected. It should be reiterated that although vaccination are likely to reduce FMDV excretion in milk, vaccinated cattle may still be susceptible to a low grade infection and shed low amounts of FMDV in the milk, potentially without any clinical signs of disease. Consequently, milk should be treated accordingly, e.g. according to EC1774/2002 (see later).

Infection and minimum doses. Susceptible livestock may be infected by FMDV as a result of direct or indirect contact with infected animals or with an infected environment. When infected and susceptible animals are in close proximity, the aerial transfer of droplets and droplet nuclei is probably the most common mode of transmission. Long-range airborne transmission of virus is an uncommon but important route of infection, requiring the chance combination of particular factors, including (1) the animal species, (2) the number and location of the transmitting and recipient animals, and (3) favourable topographical and meteorological conditions (Alexandersen *et al.*, 2003b). Studies have been carried out in animals infected by simulated natural methods (direct or indirect contact with infected donors or virus aerosols from such donors) or in animals infected by artificial methods,

including subcutaneous, intradermal, intramuscular and intravenous inoculation, intranasal instillation, and exposure to artificially created aerosols. It should be mentioned however, that studies carried out to establish minimum infective doses for the main livestock species, with various serotypes and strains of FMDV delivered by different routes, summarized in the Table below, all have an important reservation concerning the statistical significance of the numerical values as a result of the practical and cost constraints on the number of animals that could be used for the experiments and the number of variables that could be investigated. In addition, the several methods used for titration of virus were of varying sensitivity and may not be directly comparable. The results should therefore be taken as indicators and not as absolute values (Alexandersen *et al*., 2003b). The origin of FMD epidemics in countries normally free from the disease is frequently difficult to identify with certainty, but several recent outbreaks have been linked to the entry of virus in contaminated material that has subsequently been fed to animals. For example, the South Africa 2000 and UK 2001 epidemics have been attributed to the feeding of unheated waste food to pigs, and the Japan 2000 epidemic to the feeding of contaminated fodder (Knowles *et al.*, 2001; Alexandersen *et al.*, 2003a). It should be noted, that animals are relatively insensitive to experimental infection by the oral route; the dose for pigs being about 10^4 - 10^5 and for ruminants about 10^5 - 10^6 TCID50 (Sellers, 1971). These doses are much higher than those required to infect by the airborne route (Donaldson, 1987; Alexandersen *et al*., 2003b). It should also be noted, however, that animals with abrasions of the epithelium in and around the mouth may be infected by smaller doses (Donaldson, 1987). Sharp objects, such as pieces of bone, may therefore facilitate infection by contaminated waste food or facilitate the infection by other contaminated feed such as milk or milk products.

*Selected estimated minimum doses *for various species and routes of exposure*

Species	**Inhalation**	**Intradermal**	**Intramuscular**	**Nasal**	**Oral**
Cattle	10	100	10^4	10^4-10^5	10^5-10^6
Sheep	10	100	10^4	10^4-10^5	10^5-10^6
Pigs	>800	100	10^4	Unknown	10^4-10^5

*The estimated minimum doses are those reported to cause clinical disease. It is emphasized that these are not absolute values but represent estimates based on different experiments that are not necessarily directly comparable. It is possible that even smaller doses might produce infection if large numbers of animals were exposed. Doses are given as TCID50 (bovine thyroid tissue culture 50% dose end-point estimates). For further information see Alexandersen *et al.*, 2003 (Alexandersen *et al.*, 2003b). It should be noted that for intradermal and intramuscular inoculation, doses from 5 to 10 fold lower are cited in the literature, but without details of the assay systems used (Sellers, 1971).

Risk of spread by milk and milk products. As mentioned above, excretion of FMDV in the milk is likely to occur if dairy cattle, and probably also other ruminants, are infected and such milk is definitively a risk in the period before disease is confirmed. Donaldson (Donaldson, 1997) and Haas (Haas, 2003) have described the risks of spreading FMD with milk and dairy products and describes examples where such spread has happened. Sellers (Sellers, 1971) mentioned three mechanisms by which animals could potentially be infected by milk or dairy products containing FMDV: 1. Drink the milk; 2. Inhale infected droplets or aerosols generated from the milk or product; 3. The milk or dairy product may contaminate people who may subsequently handle animals.

A. Raw milk. As mentioned above, milk from affected animals may contain up to $10^{6.6}$ TCID50/ml or possibly more, as this value is based on a single finding in the field (Donaldson *et al.*, 1982). Thus, the risk of animals becoming infected if directly fed such milk is substantial, as the dose required to infect by the oral route, as indicated above, is only 10^4-10^6. Furthermore, if the milk is fed to animals with pre-existing cuts or abrasions the infective dose may be less. This is naturally only the case for untreated products in which the FMDV is likely to be rather stable provided the pH is around neutral and the temperature does not exceed 30C for long periods. However, as also mentioned above, the concentration in the raw milk is likely to be much less due to the dilution occurring during collection. Based on the assumptions given above, an average titre of FMDV in a milk tanker containing milk

from one infected herd diluted by milk from another 10 farms may be 10^1 TCID/ml milk. However, this value is based on a single finding in the field, and in certain circumstances the milk from an individual infected herd maybe significantly higher, perhaps as high as 10^5-10^6 TCID/ml milk if some animals have clinical signs but are yet not diagnosed with FMD. In this latter case the diluted milk tanker may thus contain up to 10^5 TCID/ml milk and thus easily spread FMD if fed to susceptible animals. However, in the situation where the milk may only contain around 10^1 TCID/ml milk a healthy pig may have to drink as much as 1 litre to become infected. However, if the animal has pre-existing cuts or abrasions in and around the mouth then less volume may be needed, perhaps as little as 10 ml of milk. If the milk contained around 10^1 TCID/ml and somehow was aerosolised, the dose needed to infect a calf may only be 1 ml, which appears to be a very large and unlikely amount of aerosol to be produced under natural conditions. However, if the milk contains 10^5 TCID/ml it would only require an aerosol of 0.0001 ml to infect ruminants and at this virus concentration, infection may even be transferred by contaminated people subsequently handling susceptible animals, e.g. transfer of as little as 0.001 ml of such infected milk may establish infection through damaged mucosa.

B. Milk treated by heat. High temperature-short time pasteurisation (HTST = 72C for 15 sec) is reported to give a 10^4-10^5–fold reduction in FMDV infectivity (Donaldson, 1997). However, one should take into account that the inactivation of many agents, including FMDV, is biphasic in nature and that a small fraction of virus will often survive (Donaldson, 1997). Furthermore, the thermal inactivation kinetics of FMDV may also be affected by the pH, and milk with a pH above 7 may be inactivated slower by heat that milk with a pH below 7. However, the mixing of milk from several sources at most processing sites results in raw milk of average pH around 6.5-6.8 (de Leeuw *et al.*, 1978; de Leeuw, 1980). Also, it should be realised that virus within the cellular fraction of the milk may be more heat resistant than other fractions, e.g. in the study by Blackwell et al. (Blackwell *et al.*, 1982) it is mentioned that HTST treatment resulted in approximately a $10^{3.4}$-fold reduction in infectivity of the whole milk while the same treatment of the skim milk or the pelleted cellular fraction resulted in a 10^5-fold and 10^2-fold reduction in infectivity, respectively. In the examples mentioned earlier, i.e. with raw tank milk having an initial titre of 10^1 TCID/ml or up to maybe 10^5 TCID/ml milk, after HTST treatment such milk may contain from trace amounts and up to around 10^1-10^2 TCID/ml of pasteurised milk. Although such milk has a low probability of infecting susceptible species and may require 1 litre to infect a pig, it may, if the animal has pre-existing cuts or abrasions in and around the mouth, perhaps only need as little as 10 ml of milk. Independently, if fed to many susceptible animals (Sutmoller & Vose, 1997), there may be a small but significant risk of causing an outbreak of FMD with single HTST pasteurised milk.

Ultra-high temperature (UHT) treatment (minimum treatment of 132C for at least 1 sec) is reported to cause a 10-fold higher inactivation of FMDV than HTST treatment (Donaldson, 1997), and consequently, the risk of UHT treated milk or products are likely to be reduced 10-fold compared to single HTST treated milk or products.

Heating to 80-90C for 30 sec, often used for production of milk powder, is reported to reduce the FMDV infectivity by $10^{5.4}$-$10^{6.0}$ and the subsequent drying process is likely to further reduce the infectivity (Donaldson, 1997), perhaps by a factor of 10. Thus, products treated in this way are unlikely to represent a risk in regard to spread of FMD.

Sterilisation by heat normally requires a process giving an Fc or F_0 greater or equal 3.00, which, according to EC 1774/2002 “laying down rules concerning by-products not intended for human consumption”, is equivalent of at least l2lC at the coldest point for 3 min. Such a treatment may inactivate 10^{12} spore forming pathogens, e.g. mesophilic *Clostridium botulinum* (Gaze, 2005), and is thus likely to also inactivate FMDV by a factor of at least 10^{12}.

C. Treatment by changing pH. From the literature it is clear that FMDV is only stable within a narrow pH range around 7-8. Outside this pH range the virus starts to be inactivated at an increasing speed depending on how far away from neutral. Although the exact rate of inactivation is naturally depending on many parameters, including the content of organic material and the ionic strength of the product, a guiding rule of thumb is as follows: FMDV half-life, or under optimal conditions the 10-fold reduction time, equals roughly 12 hours at pH 6.5; 1 min at pH 6; and 1 sec at pH 5 (Fellowes,

1960; Bachrach, 1968). Thus, the effect of lowering pH will depend on the actual pH achieved and the time of the treatment. However, it should be remembered, as mentioned above under heat treatment, that also pH dependant inactivation of FMDV is biphasic in nature and that a small fraction of virus will often survive for a longer period. In milk products in particular, the exact quantitative effect on virus infectivity of lowering pH may be difficult to assess. Sellers (Sellers, 1968) reported a $10^{4.5}$->10^{5} log reduction of FMDV titre after lowering pH to 6 for 30 min at 4C unless the material contained milk (40%) after which he only found a $10^{2.2}$ log reduction in titre. However, this being said, long term infectivity of FMDV, even in meat, is unlikely to occur at a pH level of under 6.2 (Henderson & Brooksby, 1948).

D. Drying. The effect of drying can be difficult to quantify exactly and will depend on the actual process involved. The drying itself is likely to reduce the FMDV infectivity by a factor of 10-fold or more, however, the drying process often includes some initial heat treatment to facilitate the drying process adding to the inactivation and thus often result in a further reduction in infectivity. In 2003/85/EC on "Community measures for the control of FMD", it is specifically mentioned that the drying process should again heat the product to at least 72C and as mentioned above, such products often involve heating the product to 80-90C for 30 sec.

B. Combination treatments. Combination treatments may consist of repeated heat-treatments, e.g. double HTST or HTST followed by UHT treatment. As an example, Donaldson (Donaldson, 1997) mention the situation during the outbreaks of FMD in Denmark in 1982, where a treatment consisting of initial HTST treatment was followed by 80C for 3 sec and, in some cases, by lowering pH to below 4.5 (Danish Veterinary Service, 1982a; Danish Veterinary Service, 1982b). It was estimated that 18 million kg of milk treated in this way was fed to domestic animals during the epidemic without causing any outbreaks (Danish Veterinary Service, 1982a; Danish Veterinary Service, 1982b). Other possibilities of combination treatments often used are heat treatment followed by lowering pH or drying. Various EU regulations describe such treatments, e.g. EC1774/2002 "laying down rules concerning by-products not intended for human consumption" in which it for milk and milk-based products is mentioned that such products should be first treated by HTST pasteurisation followed by a drying process for dried milk or dried milk products, and for acidified milk products, a process by which the pH is kept below pH 6 for at least an hour. In regard to milk or milk-products from third countries, EC1774/2002 stipulates that the same treatment, or double heat treatment, may be used from FMD free regions while milk and milk-based products from third countries or regions where FMD have been present within the last 12 months should undergo either the same treatment or double heat treatment or a sterilisation process to an Fc>3. Similar treatments are also described in the Foot-and-mouth disease directive 2003/85/EC which for milk and milk products not intended for human consumption and milk and milk products for animal consumption, mention a sterilisation process to an Fc> 3, double HTST pasteurisation, or HTST or UHT followed by a drying process (it is specifically mentioned that the drying process should again heat the product to at least 72C and as mentioned above, such products often involve heating the product to 80-90C for 30 sec) for dried milk or dried milk products and for acidified milk products, a process by which the pH is kept below pH 6 for at least an hour. The combined effects of the mentioned treatments is likely to be a 10^{6}-fold or more reduction in FMDV titre and additionally, either have the added safety of relying on two different principles of inactivation or at least a repeated process. Consequently, products from such combination (double) treated milk or milk-products are unlikely to spread FMD. It should be noted however, that although treatment of products at pH 6 for an hour results in a reduction in the potential infectivity content, it is wise, as indicated in the SCAHAW Report on "Strategy for Emergency Vaccination against Foot and Mouth Disease (FMD)" of 10 March 1999 for feeding of whey to pigs, that the pH treatment is rather prolonged, assuring that the product is kept or transported at the lower pH for several hours (rather than only 1 hour) to ensure full inactivation.

F. Risk of post-processing contamination and possibility to survive during transport and storage. EC1774/2002 "laying down rules concerning by-products not intended for human consumption" lists the safeguards necessary for safe processing of milk and milk-based products: every precaution must be taken not to contaminate the products and the final product must be packed in new containers or transported in vehicles or containers that have been disinfected and approved. Thus, the risk of

contamination is likely to be low and would most likely, if it happened, only result in a very low level of infectious FMDV in the finished product. Nevertheless, it is of utmost importance that treated products are adequately shielded from subsequent contamination. Any infectious FMDV not inactivated during the processing may be relatively stable during storage and transport if the pH of the product is around neutral and the temperature is not above 30C for extended periods. Similarly, any infectivity still present in dried products is likely to be stable.

Summary of main uncertainties. Main uncertainties in relation to the risk of milk and milk can be summarised as below and could individually or combined be the target of specific studies:

- Minimal dose to infect - may give a 10^3-10^4 fold level of uncertainty in animals with cuts or abrasions
- Excretion levels in milk, prevalence on farms and number of farms affected at time of reporting - may give a 10^3-10^4 fold level of uncertainty at the herd or area level

- Uncertainties about heat treatment:
    - * Cellular fraction more stable - 10^3 fold uncertainly
    - * Biphasic inactivation
    - * pH dependant
    - * UHT inactivation not well described
- pH inactivation - biphasic, small fraction may survive
- Drying - not well described by itself

- May be differences in the stability of various serotypes or subtypes
- Even less is known about other species than cattle

Discussion

As evident from the review of known data given above, and already mentioned at the 2003 meeting in Gerzensee, Berne, Switzerland in September 2003, the scientific literature on FMDV inactivation kinetics under relevant conditions is fragmented and with major inherent difficulties for making quantitative comparisons on the data from various sources and of various quality and statistical significance. As explained by Have (Have, P. An assessment of guidelines for treatment of meat from a FMD vaccination zone. Session of the Research Group of the Standing Technical Committee, European Commission for the Control of Foot-and-Mouth Disease 2003: 149-152), the effect of heating is detennined by a combination of time and temperature and the inactivation kinetics are often assumed to be first-order although this does not appear to be the case for FMDV inactivation and consequently, tailing effects should clearly be considered. The decimal reduction time D_T is the time needed to reduce the viable population by 90% at the temperature T. Semi-log plots of D-values against temperature yield near linear relationships, from which estimated z-values can be calculated as the number of degrees temperature required to change D by one log unit. Heat treatment includes a heating phase and a cooling phase and to account for the combined effect during heating and cooling, the temperature/time relationship data can be used to calculate lethal rates over the entire process and integrating into a cumulated lethal effect, expressed relative to a standard treatment at a chosen reference temperature and taking advantage of knowing the value of z (Peleg, 2003).

Conclusions

Clearly, detailed studies of FMDV inactivation in milk and milk products from infected animals should be supported and designed to provide D and Z-values in milk and relevant milk products and should also provide additional evidence on the quantities of FMDV excreted in the various fractions of milk from infected animals and the minimum doses to infect.

Recommendations

- A small working group should urgently draft detailed plans for studies of FMDV inactivation in milk and milk products from infected animals and funding and input from industry should be sought.

- The studies should be designed so they can provide D and Z-values of FMDV inactivation in milk and relevant milk products. Special attention should be put on the inactivation kinetics in the cellular fraction versus the aqueous (skim milk) and cream fractions and also particular attention should be put on selected treatments such as HTST (72C for 15 sec), UHT (132C for at least 1 sec), 70C, 80C, 90C, drying and pH.
- Clearly also other conditions apart from the temperature, such as product type, pH, ionic strength and a number of other factors are also of importance for the kinetics of inactivation and should be an integrated part of the studies as should established combination treatments.
- The studies should be complementary to existing knowledge and done under conditions making comparison to earlier and coming studies feasible.
- The studies should also provide additional evidence on the quantities of FMDV excreted in the various fractions of milk from infected animals at various stages of the infection.
- Studies looking at the actual dose needed to establish infection by the oral or aerosol route by infected milk is also needed, in particular in animals with abrasions or cuts in the oral epithelia.
- Relatively large animal experiments are needed in order to provide statistically significant data.

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Appendix 24

FMDV Survival in meat
Required input from a risk assessment point of view

Matthias Greiner and Thomas B. Jensen (not at DFVF anymore)
Danish Institute for Food and Veterinary Research

Background

EUFMD RG has requested the DFVF risk assessment group to share compiled information about the FMDV survival in meat products. The information below is taken from an ongoing study "Risks associated with imported meat from swine vaccinated against foot and mouth disease virus. A qualitative assessment". Briefly, the scope of this risk assessment was a description of risk scenarios in the context of the new vaccination policy for FMD given by Council Directive 2003/85/EC of September 29th 2003. Only vaccination in swine was considered in the assessment. The assessment was of qualitative nature as the legal and illegal marked response in the trade of meat from vaccinated swine in European is impossible to be predicted.

The risk assessment framework

The release assessment is part the formal risk assessment process according to OIE standards. It describes the scenarios for introduction of infectious virus into a country by one or many specified introductory pathways and assesses the probability of any of these pathways to result in a release. The release of virus from imported meat from vaccinated animals depends on the vaccine efficacy, the presence of virus in the tissues at the time of slaughter and the survival of virus during the processing and transport.

Survival of FMDV in non-cured meat

The literature on virus survival in animal products has quite miscellaneous focus points, and nothing that directly relates to the definition of meat as used in the EU-legislation. Definitions of products of animal origin is described in Council Directive 64/433/EEC of 26 June 1964, in which the exact definitions of meat (articles 1 and 2) was latest changed by directive 83/90/EEC of 7 February 1983. In the revised text, five points of article 2 (a, b, d, e and f) are relevant for the definition of fresh meat:

(a) "Meat", means all parts of domestic bovine animals (including buffalo), swine, sheep, goats and solipeds which are suitable for human consumption;
(b) "Fresh meat" means meat, including meat vacuum-wrapped or wrapped in a controlled atmosphere, which has not undergone any treatment other than cold treatment to ensure preservation;
(d) "Carcase" means the whole body of a slaughtered animal after bleeding, evisceration and removal of the limbs at the carpus and tarsus, removal of the head, tail and the udder, and in addition, in the case of bovine animals, sheep, goats and solipeds, after flaying;
(e) "Offal" means fresh meat other than that of the carcase as defined in (d), even if it remains naturally connected to the carcase;
(f) "Viscera" means offal from the thoracic, abdominal and pelvic cavities, including the trachea and oesophagus;

This implies that fresh meat of pigs can consist of any protein tissue (normal understanding of meat), fat, skin, bone, lymphoid tissues and residual blood in larger vessels.

The literature has some information with regard to post-mortem survival of FMDV in animal tissues. However the data was generated in a time, when implementation of the information in risk assessment was not an issue, and consequential that structure of the published data may not be particularly helpful. Moreover, the published data fall into two major categories. One category of data presented as

survival times of FMDV in single tissues and one category of data presented as FMDV survival in meat cuts (i.e. multiple tissues). An overview of the literature on the subject is given in Tab. 1.

Survival of FMDV in cured meat products

The virus survival of FMDV has been investigated in various cured products. Under an assumption that none of the curing processes enhances virus survival of FMDV, the numbers presented in Tab. 2 should be accepted as minimum values for FMD virus survival in theses tissues irrespective of whether the meat was cured or not.

The effect of pH in meat of cattle and pigs

The pH value in infected animal products has long been recognised as an important factor for survival of FMDV. The pH drop is assigned to lactic acid arising from anaerobic post-mortem metabolism, and thereby dependent on the glycogen reserves in the tissues at the time of slaughter. This largely restricts the pH drop to the pure muscle tissue with only minor distribution to surrounding tissue due to compartmentalisation by connective tissue. It has been found that pH in large lymph nodes was unaffected by the pH drop in the adjacent muscle. The post-mortem drop in muscle pH is relatively fast. It has been hypothesised that any survival of virus in muscle tissue should be referred to the minute amount of blood in the capillaries - which is subsequently subject to acidification from the surrounding muscle tissue. The inherent hypothesised difference between blood in capillaries on one side and other tissues and blood in larger vessels on the other side in their sensitivity to acidification should be explained by the physiological capacities of capillaries with regard to exchange of gas and metabolites. The effect of pH in meat is supported by the fact that FMDV survival in minced meat is reported to be zero, and the instant disappearance of virus is thought to be caused by redistribution of the lactic acid across any compartments existing prior to mincing. Finally the pH effect in pure meat is reflected in the set-up of models used for studying of heat and other treatments.

An anecdotal substantiation of the pH effect was provided in the study of (Henderson and Brooksby, 1948), who had to thaw their samples of frozen meat in a buffered solution in order to recover virus. This study indicated that the pH drop is reinitiated after thawing if the meat is frozen immediately after slaughter.

It should be noted that the drop in pH provides a progressively more unfavourable environment for virus survival, and absence of drop below 6.0 should not be interpreted as certain virus survival. This mechanism renders a cut-off for survival of FMDV partially meaningless and is reflected by the lack of consensus in the literature. Cut-off values between 5.9 and 6.5 have been encountered.

Standard comparator muscles

Deboned and matured beef is considered safe with regard to transfer of FMDV. This is exemplified by the requirements for trade of beef in the directive 2003/85/EC, where trade of deboned fresh beef, matured at >2°C for >24 hours to a pH in the middle of the *Longissimus dorsi* muscle below 6.0 (annex VIII) is generally allowed from 30 days after the last vaccination in the vaccination campaign (article 58-8). This establishes *Longissimus dorsi* as a standard comparator muscle of beef with regard to pH, and there seems to be consensus that all other muscles should possess the same the same virucidal pH. The term ‘standard comparator muscle’ is an expression more than a fixed standard, since the choice of standard comparator muscles may vary between studies and other contexts such as control purposes.

No such standard seems to exist for pigs in the context of infectious diseases. If, however, the scope is turned towards the meat science literature, there is an abundance of literature reporting pH values in pig meat. For example, (Berg et al., 2003) found no differences in pH 24 hours post-mortem across 48 pigs in four treatment groups. They investigated the pH in three different muscles *(M semimembranosus, M. Longissimus (dorsi?) and M Gluteus medius)* of each pig and the means was in

the range of 5.3 to 5.4 with a variability described by a maximum standard deviation of 0.1 (S.E.x square root of N = $0.031x12^{0.5}$).

A pH between 5.6 and 5.8 24 hours post-mortem in loin *(M. Longisimus dorsi)* was found in Italian heavy pigs (Corino et al., 2003). The post-mortem pH development in *M. Longissimus lumborum* and *M. Biceps femoris* has been found in the range of 5.4-5.6 (Lambooij et al., 2004). The ultimate pH (at least 24 hours post mortem) in a larger number of studies was reviewed and it was found that the majority of results indicated ultimate pH values below 6.0 in pig meat (Bendall and Swatland, 1988). However, exceptions have been reported. One cause of the exception was variation between muscles. In one study of 2893 pigs reported variation in mean of ultimate pH between the four standard comparator muscles in that study with mean ultimate pH of 5.75, 5.86, 6.14 and 6.15 (Gallwey and Tarrant, 1978). Besides the within animal (between muscle) variation of pH, also between animal and between race variation has been reported.

At least four different profiles exist for post-mortem metabolism in meat of Danish Landrace pigs. One did, despite an apparent pH drop, only reach ultimate pH between 5.7 and 6.3 in the standard comparator muscle of that study (Briskey and Wismer-Pedersen, 1961). Due to the age of the study, the fraction of pig carcasses with that profile must be considered uncertain in relation to the Danish situation of today - and in relation to the genetic constitution of pig production in other countries. In a recent study of 165 Danish Landrace crossbreeds and 190 Duroc crossbreeds, it was found that pH of *M. longissimus dorsi* was in the range 5.32 to 5.88 with a mode (>50% of observations) in the interval 5.54-5.67 for both races after 24 hours (Lindahl 2004, personal communication). The between-race variation can e.g. be reviewed (Bendall and Swatland, 1988). It appears that same breed may have varying mean ultimate pH in the same standard comparator muscle. Thus, it may be that the between-race variation has a subcomponent of between-lineage variation. It has previously been hypothesised that stress related glycogen depletion prior to slaughter may cause less post-mortem drop in pH - and possibly account for some of the between-animal variation seen. However, the effect of stress prior to slaughter had no effect (van der Wal et al., 1999).

Discussion

The knowledge about FMD virus survival in beef is extensive whereas data for pork and other swine meat products are sparse and mostly related to specific products. The experiments have typically been conducted on small sample sizes. The experimental design (number of animals per group, virus type, viraemic concentration in tissues, processing) is necessarily very context-specific. The biostatistical treatment of the published results, according to our impression, has not been to the standards of food hygiene or pharmacological research. In effect, the use of published figures in risk assessment models (qualitative or quantitative) introduces uncertainty because the results may not apply to fresh meat in general or to the speciality product under consideration. Furthermore, the statistical uncertainty (measurement error and biological variability) is not yet well represented in our risk models.

The question needs to be addressed whether vaccination is an important biological factor in these models beyond its impact on virus concentration in blood and tissues.

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Table 1. Virus survival for FMDV in non-cured products and tissues of pigs and cattle

Tissue (origin), number of animals	FMDV survival in days last pos./first neg.	Source
Bone marrow (pig)	42 / ?	Tab. 8 in (Cottral, 1969)*
Muscle (pig)	/ 1	(Cottral et al., 1960)
Blood on surface of carcass (pig)	4 / ?	(Cottral et al., 1960)
Blood clot in heart (pig)	34 / ?	(Cottral et al., 1960)
Lymph nodes and blood (pig)	70 / ?	(Cottral, 1969)
Lung, kidney and spleen (pig)	42	Tab. 9 in (Cottral, 1969)*
Stomach and tongue (pig)	10	Tab. 9 in (Cottral, 1969)*
Brain (pig)	17	Tab. 9 in (Cottral, 1969)*
Muscle (pig), N=2	1 (70) 1 d: Refrigerated meat, 70 d: Frozen meat	(Savi et al., 1962)
Blood, brain, lung, bone marrow, lymph nodes, stomach, intestines, tongue, fat and parotid (pig), N=2	10 (210), 10 d: Refrig. tissues, 210 d: Frozen tissues	(Savi et al., 1962)
Spleen, kidney and liver, N=2	1 (210), 1 d: Refrig. tissues, 210 d: Frozen tissues	
Bone marrow (cattle)	80 / ?	
Bone marrow	194 / fin	(COX et al., 1961)
Bone marrow (cattle), N=8 ?	210 / ?	(Cottral, 1969)*
Lymph nodes (cattle)	120 / ?	(Cottral, 1969)*
Hemal nodes (cattle)	120 / ?	(Cottral, 1969)*
Tongue and cheek (cattle)	33 / ?	Tab. 8 in (Cottral, 1969)*
Intestines (cattle)	6 / ?	Tab. 8 in (Cottral, 1969)*
Muscle (cattle), N=2	1 / 3, (60 / fin), Positive reactions at 60 days were thought to be due to contamination.	(Cottral et al., 1960)
Bone marrow, N=2	73	(Cottral et al., 1960)
Blood and lymph nodes (cattle), N=2	60 / fin	(Cottral et al., 1960)
Synovial fluid (cattle)	19	(Gailiunas et al., 1960)
Blood, lung, bone marrow, lymph nodes, rumen, tongue, parotid and uterus (cattle), N=2	8 (210), 8 d: Refrig. tissues 210 d: Frozen tissues	(Savi et al., 1962)
Liver and spleen (cattle)	1 / 2	(Cottral et al., 1960)

* Contains references to original work.

Table 2. Virus survival in different tissues of cured products of pigs *(cursive = cited ~ unverified information)*

Reference	**Curing**	**Product**	**FMDV survival in days**	**Comment**
(Mebus et al., 1993)	Iberian	Ham-muscle	0[a,b] (<14)[c]	P(0 pos at day 14| prev at day 0) = 0.91
	Iberian	Shoulder-muscle	0 / 14	
	Iberian	Loin-muscle	0 / 14	
	Serrano	Ham-muscle	0 / 14	
(McKercher et al., 1987)	Parma	Ham-muscle	÷[b] (<72h)[c]	American experiment
	Parma	Ham-muscle	0[b] (<30h)[c]	Italian experiment
(Savi et al., 1962)	-	Ham-muscle	25	
(Mebus et al., 1993)	Iberian	Ham-fat	0[a,b] (<14)[c]	P(0 pos at day 14| prev at day 0) = 0.29
	Iberian	Shoulder-fat	0[a,b] (<14)[c]	
	Serrano	Ham-fat	140[d]	
(McKercher et al., 1987)	Parma	Ham-fat	0[b] ~ 72h (<30)[c]	American experiment
	Parma	Ham-fat	96[b]	Italian experiment
(Dhennin et al., 1980)		Ham-fat	176 / 183	
		Shoulder fat	155 / 169	
(Savi et al., 1962)	-	Ham-fat	46	
(Mebus et al., 1993)	Iberian	Ham-bone marrow	56	
	Iberian	Shoulder-bone marrow	84	
	Serrano	Ham-bone marrow	84	
(McKercher et al., 1987)	Parma	Ham-bone marrow	30[b]	American experiment
	Parma	Ham-bone marrow	30[b]	Italian experiment
(Savi et al., 1962)	-	Bone marrow of hams	89	
(Mebus et al., 1993)	Iberian	Ham-In. popliteus	112	
	Serrano	Ham-In. popliteus	168[d]	
(Cottral et al., 1960)		***Bone marrow of hams***	*183*	
(Dhennin et al., 1980)		*Salted bacon*	*183 / 190*	cited in (Mebus et al., 1993)
(Savi et al., 1962)		Bacon	10	Tab. 10 (Cottral, 1969)
(Panina et al., 1989)		Different Italian salamis	0[b] (<72h)	

[a] Only 2 of 62 pigs had virus in meat on the day of slaughter.
[b] The last virus positive sample
[c] The first virus-free sample - only included for samples negative
[d] Reported to be associated with hemorrhagic specimen

Appendix 25

Risk assessment on Foot-and-Mouth Disease (FMD) in pork from vaccinated animals

E. LOPEZ, A. DEKKER, M. NIELEN

Abstract

Foot-and-Mouth Disease (FMD) is a problem of great economic importance in livestock producing countries. Time and again, it has been experienced in a number of outbreaks worldwide. The Type O strain, for example, has been noticeable in recent outbreaks in most of Asia, Europe, and South America affecting susceptible pigs, sheep and cattle in these regions. Spread of the FMD virus is mostly by direct or indirect contact with infected animals and by airborne transmission. The inadvertent feeding of infected, inadequately processed or untreated meat and meat products also make the livestock population vulnerable to FMD (Alexandersen and others 2003).

The disease causes much astir in international trade, particularly in Europe, where borderless economies can be greatly affected. Free, unrestricted trade in the European Union (EU) as well as the close proximities and accessibilities of member states open avenues for the unhampered spread of the disease. The 2001 FMD epidemic in Great Britain, for example, quickly gave rise to subsequent epidemics in France, Ireland and The Netherlands (Bouma and others 2003).

In the case of the FMD epidemic in The Netherlands, immediate culling of affected animals, emergency vaccination, slaughter and destruction of vaccinated animals was done to control the disease in addition to culling of all susceptible animals in affected farms. Pre-emptive culling in nearby farms within a one kilometer radius of these farms (EEC Council Directive 85/511/EEC) was carried out. Emergency vaccination followed as a further control measure, that is, suppressive vaccination was performed from a radius of two kilometers around an affected farm going inwards (EEC Commission Decision 2001/246/EC). But suppressive vaccination also meant that all vaccinated animals were to be destroyed (Pluimers and others 2002). With the subsequent slaughter of many vaccinates, comes the problem of disposing their carcasses. The volume of meat from these carcasses is more than what local domestic consumption can handle. Considering putting into good use the surplus of meat from vaccinated animals, marketing this commodity can be an option. But what are the risks that have to be dealt with to safely trade this commodity within the EU?

Work had been done on the risks of FMD in various commodities in international trade (Callis 1996, Metcalf and others 1996, Gallagher 2002, Sutmoller and Casas Olascoaga 2003). Risk analyses and assessments on the importation beef (Astudillo and others 1997, Yu and others 1997, Sutmoller 2001) and milk and dairy products (Heng and Wilson 1993, Donaldson 1997) from countries infected with FMD were carried out. However, no studies have been done yet on the risks posed by FMD virus in pork or its meat products, particularly not from vaccinated pigs.

This paper assesses the risks associated with FMD virus in pork from vaccinated animals in FMD-affected areas already declared free from the disease. Possible risks of FMD virus contamination from pork of vaccinates are identified (farm to abattoir) and their probabilities are determined in a semi-quantitative risk assessment. This paper also makes a qualitative risk

assessment of the processed meat and its meat products to further evaluate the potential risks of exporting pork to FMD-free countries within the EU.

SEMI-QUANTITATIVE RISK ASSESSMENT

Scenario tree

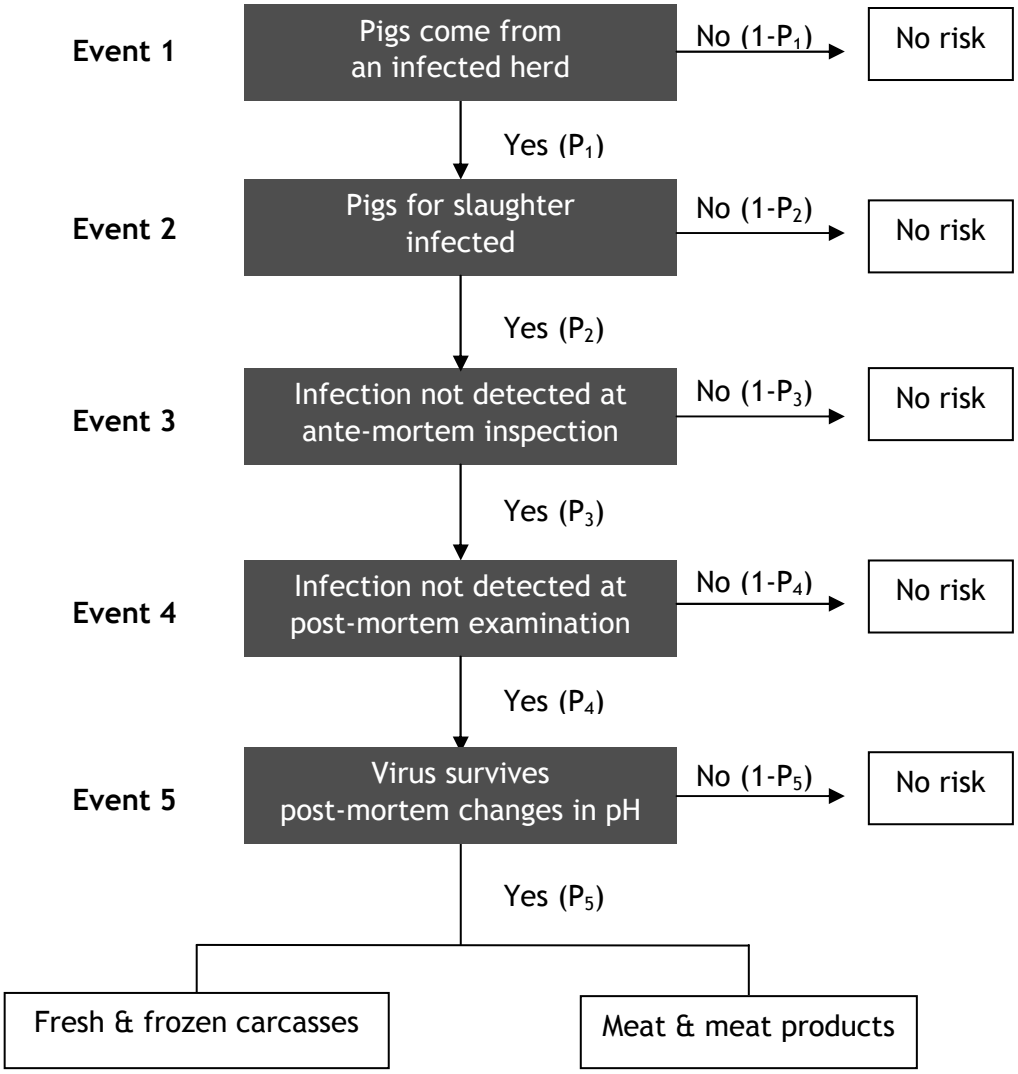
Fig 1 shows the scenario pathway for the possible risks of contaminating pork with FMD virus. It is assumed that the sources of animals are vaccinated pigs coming from an area which has regained freedom from FMD.

In the articles of a new EU directive (EEC Council Directive 2003/85/EC), three phases of a waiting period are described till an affected area is declared FMD-free (Fig 2). Phase 1 is, at least, a 30 day elapsed period after the completion of emergency vaccination. Phase 2 follows this period where a survey and a classification of holdings are completed. Phase 3 is the time after the completion of the survey and the classification of holdings until the foot-and-mouth disease and infection status is recovered. In this paper, the risk assessment is carried out for Phase 3 only, because it seems unrealistic to restart trade in Phase 1 and 2.

A semi-quantitative risk assessment from Events 1 to 5 is made using the software program "@Risk" version 4.5 (©2002, Palisade Corporation) to determine the probability distributions for the events in the scenario tree and sensitivity analysis. The combined risk of having contaminated meat is computed by multiplying all the estimated probabilities of the five events.

After the last event, a qualitative risk assessment is made as regards the possible risks of the FMD virus still surviving in fresh, frozen carcasses and meat and meat products for export.

FIG 1: Scenario pathway for the risk of contaminating pork with FMD virus



Probability of Contaminated Meat (Pcontam) = $P_1 \times P_2 \times P_3 \times P_4 \times P_5$

The events where these risks can be evaluated are identified as follows:

- **Event 1** - the source of the vaccinated pigs come from an undetected, infected herd or farm
- **Event 2** - the selected vaccinated pigs are infected at the time of slaughter
- **Event 3** - apparent infection in the live, vaccinated pigs is not detected during ante-mortem inspection
- **Event 4** - meat inspection fails to detect infection during post-mortem examination
- **Event 5** - the FMD virus survives post-mortem changes in pH in meat

FIG 2: Schematic Diagram of the Phases of the Waiting Period for FMD

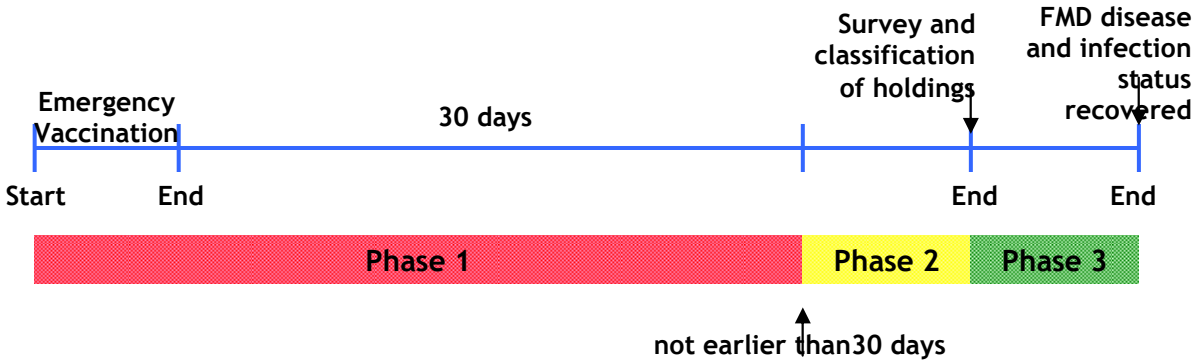


TABLE 1: Estimated phases of the outbreaks in the 1997 FMD epidemic in Taiwan

Dates	Cumulative no. of days	No. of newly-infected herds	Herd Prevalence	Remarks	Phase in waiting period
March 14-20	6	28	0.110%		
March 21-27	12	689	2.713%		
March 28-April 3	18	850	3.346%	Started mass Vaccination (March 29)	Phase 1
April 4-10	24	1,087	4.280%		
April 11-17	30	1,113	4.382%		
April 18-24	36	772	3.039%		
April 25 - May 1	42	625	2.461%		
May 2-8	48	677	2.665%	Started blanket Vaccination (May 3)	
May 9-15	54	240	0.945%		Phase 2
May 16-22	60	42	0.165%		
May 23-29	66	15	0.059%		
May 30 - June 5	72	5	0.020%		
June 6-12	78	0	0.000%		
June 13-19	84	1	**0.004%**		Phase 3
June 20-26	90	0	**0.000%**		
June 27 - July 3	96	0	**0.000%**		
July 4-10	102	2	**0.008%**		
July 11-15	108	1	**0.004%**		
July 16-23	114	0	**0.000%**		

TABLE 2: Summary of probabilities for each event in the scenario tree

	Event		Probability	Reference for values
1	Pigs come from an infected herd	P_1	0.0016%	Yang and others 1999
2	Pigs for slaughter infected	P_2	0.47%	Chung and others 2003
3	Infection not detected at ante-mortem inspection	P_3	79.7%	Metcalf and others 1996
4	Infection not detected at post-mortem examination	P_4	79.7%	Metcalf and others 1996
5	Virus survives post-mortem changes in pH	P_5	1.1%	Metcalf and others 1996

TABLE 3: Calculated volume of exports based on the risk of exporting a contaminated product by the Netherlands (2003 figures)

	Export Volume	Quantity	
Commodity	metric tons	metric tons	kilos
Pork	343,657	0.00044	0.43925
Bacon	129,568	0.00017	0.16561
Sausages	34,033	0.00004	0.04350
Meat Preparations	20,995	0.00003	0.02684

Discussion

There is little data available on surveillance and monitoring programs for vaccinated pigs after an FMD outbreak. More studies should be made on the FMD antibody titers using ELISA-based tests detecting NSPs to differentiate infection from vaccination.

In a global sense, the prevalence rates used in the semi-quantitative risk assessment here may not be representative of the true picture of the disease in a vaccinated animal population. Disease situations differ from region to region. In Europe, outbreaks in pigs are smaller, containment is better therefore the risks are much lower. Nevertheless, this study would necessitate for more scientific literature depicting different scenarios of FMD disease prevalence in other countries.

Regardless of the present prevalence of FMD, residual virus survivability and infectivity in pork depends mainly on the chemical changes that take place at post-mortem. The sufficient lowering of pH in muscle tissues of carcasses is a crucial factor in the inactivation of the virus. It is important, therefore, that meat manufacturing or processing systems observe required chilling times after slaughter to hamper further survivability of the virus.

Where there are organ systems which are unaffected by these pH changes, the FMD virus is most likely to survive. Fresh and frozen carcasses pose potential risks in FMD virus

introduction since these can harbour the virus in remaining lymph nodes, residual blood, bone marrow and the skin. In the case of meat and meat products, the risks are much lower, especially those which do not contain bone. Various meat processing methods like cooking with required heat treatments, long curing periods and specific processing methods (e.g., grinding), can contribute to the eventual inactivation the virus.

Information on infectious virus survival in the skin, lymph nodes, bone marrow and blood of already vaccinated animals are lacking. More studies in the pathogenesis of the virus in these particular organ systems in a vaccinated population especially after an outbreak is therefore warranted.

Appendix 26

Foot-and-mouth disease in wildlife
Risks and risk management proposals for Europe

François Moutou

Introduction

The real importance of many wildlife species in foot-and-mouth disease (FMD) epidemiology is quite often addressed but the answers are not so easy to find or to use. The questions are many, combining free-ranging or captive species and animals, but also items like passive carrier, active carrier, biological reservoir, as well as receptivity, susceptibility, clinical expression, long-term carrier, for many different species belonging to quite distinct zoological groups. The possible differences in the answers depending of the virus types or sub-types involved must not be forgotten.

Depending of all these items, the possible risk linked to any species, under any circumstance, could be important to evaluate. The easiest approach however is to ask the questions following zoological groups. Short synthesises are here proposed, written following the references mentioned in the bibliography. For each situation, the conclusions should be read through their own context: free-ranging species, farmed species, and zoological gardens species. The arguments here proposed are those of a risk management point of view, linked to known FMD outbreaks identified in a European country, and possibly involving "non classical species". The proposals could be included in contingency plans. This is not a review on FMD in wildlife in Europe.

It must be noted that owners of some of these species when captive, or even managers of native wild species, may be less informed than farmers on FMD risks, consequences and policy. Their reactions will also be different. Other elements that just economical factors could be used and this could make the discussions more difficult.

1 - Backgrounds

Classically, FMD is associated with mammalian species of Artiodactyla order but textbooks still give a rather longer and rather heteroclite list of susceptible species. In fact, many references still in use are rather old and are often just linked to clinical observations. Not always is or was a confirmation performed with FMD virus isolation and identification mentioned. This does not mean that all is to be rejected but certainly a more critical review should be necessary and the data revisited. This was clear during the 2001 European outbreaks when some zoological gardens managers or exotic species (camelidae for instance) breeders were asking for derogations (for instance vaccination or non-culling strategies) to the actual European legislation for their own animals. So the point here is also to imagine suggestions adapted to these situations within the frame of the application of 2003/85/EC directive, as it is possible.

The order of presentation of the zoological groups is rather linked to systematics than to their real or possible epidemiological importance. It must be added that free ranging species may, as least in theory, act as passive vectors of the virus during an outbreak, but this is not only true for wildlife. This will only be mentioned with birds.

2 – Birds

It may be a little surprising to speak of birds, as they do not seem to be at all susceptible to FMD virus. In fact, this question had already been asked for many years, birds being seen then just like passive carriers of the virus, possibly over long distances.

Speaking of migratory birds, it is possible to say that billions of them are crossing twice a year the Mediterranean Sea, both ways. Not a single outbreak of FMD linked to SAT types virus ever occurred in Europe or in northern Asia after the spring migration.
During the British epidemic of 2001, racing pigeons were indeed forbidden over the Channel but this was more for global biosecurity reasons.
Researches have been conducted on this topic however not recently (Eccles 1939) and were not conclusive. A quite recent document tries to revisit this hypothesis but the references and the arguments are using typical bird-adapted virus like West Nile virus or orthomyxoviridae, when FMD virus is clearly not an avian-adapted virus (Koch *et al*. 2001). It does not seem that this manuscript has been published.

So, it is sufficient to say here that, up to today, it has been possible to handle all documented FMD outbreaks without any specific action against bird life.

3 – Hedgehogs (Mammals Insectivora)
Insectivora mammals, in fact mainly the Western European hedgehog (*Erinaceus europaeus*), are often mentioned as being able to carry FMD virus locally around an outbreak. An ancient citation (McLaughlan and Henderson 1947 [not seen]) speaks of animals naturally infected closed to outbreaks in Great Britain. In 2001, the survey forms used in British infected premises had a specific question to address the point of hedgehogs known to be present, or not, on the farm. This argument is in use nowadays in some USA states to ban any hedgehog as pets (Riley and Chomel 2005). In this case it must be recalled that hedgehogs are only known in the Old World, so, in the USA, they have to be imported from somewhere else. Apparently, commercial hedgehogs farms do exist there, but probably not with the European species. The most common one should be an African Hedgehog, *Ateletix albiventris*, locally called « pygmy hedgehog » as it is a little smaller than the European species. It is however possible to find exotic (Africa, Asia) hedgehogs species, including this one, on sale in pet shops in Europe. Law in Europe protects both native species, the Western and Eastern European hedgehogs.

Here too, it is possible to say that documented FMD outbreaks were put under control without any specific actions against hedgehogs. Of course, in what is called « neighbourhood risk », if cats as dogs or domestic pigeons are included, then why not hedgehogs or domestic sparrows?

4 – Elephants (Mammals Proboscidae)
Elephants do not belong to European fauna but on one hand lesions linked to FMD were described in both species, Asian as well as African elephants, and on the other hand these animals are often presented in zoological gardens or even in circuses in Europe. It can be added that they represent a real value, not only just economical.

FMD clinical descriptions are more numerous for the Indian elephants (*Elephas maximus*), which may be not only linked to the fact that they can be domesticated and tamed but also to a higher specific susceptibility (Pyakural *et al*.1976, Hedger and Brooksby 1976). The Asian species seems to be able to do a natural disease even if viral transmission from a sick elephant to any other animal does not seem to have been documented. However, transmission between elephants is possible. In Africa, most of the data for natural situations come from South Africa. Out of 3535 elephants from Kruger National Park (RSA) culled and examined during 4 years during the 1960s and 19670s, none was showing a single lesion or even a sign looking like FMD when it is well known that the virus is present and circulating in local wildlife (African buffaloes) in this National Park (Hedger and Brooksby 1976). At the end, thousands of wild elephants have been culled and examined between 1968 and 1994 with the same negative results, and thousands of serologies have also been performed, all negative (ProMED 20001123.1908). The only description of an apparently spontaneous clinical disease in an African elephant came for the years 1970s, in an Italian circus. In fact, it could have been an Asian elephant instead, as the other species is really rare in circus (ProMED 20001123.1908) !

Under laboratory conditions, the African elephant (*Loxodonta africana)* may show severe lesions when the virus is experimentally inoculated to a young animal, but the species does not seem to be able to transmit nor to carry the virus. It makes no antibody and cannot get infected by contact with sick cattle. A young elephant which received a SAT2 virus strain in its tongue in 10 different places showed no reaction, neither clinical nor serological and it has not been possible to re-isolate the virus afterwards. The initial viral concentration of the inoculate is not known (Hedger *et al*. 1972). Another study describes a clear clinical disease in young elephants after they were infected by the virus (Howell *et al*. 1973 [not seen], Pinto and Hedger 1978, ProMED 20001123.1908).

So, elephants have only a very low epidemiological importance, knowing also that a recently described herpes virus in these species gives lesions looking like those of FMD in captive individuals and may have been responsible of some of the lesions associated with FMD, at least in captivity.

5 – Suidae (Mammals Artiodactyla)
As the wild boar is the wild ancestor of domestic swine, it is true that they share receptivity and susceptibility to quite a lot of pathogens. However, the recent FMD outbreaks that occurred within Europe have shown that the transmission of the virus between the wild and the domestic stocks was not so common. In 2001, out of 208 free ranging wild boars tested in the Netherlands, all gave a negative serological result (Elbers *et al*. 2001). If there have not been so many papers published on this topic in the UK, it may be linked to the fact that the species vanished from the country some centuries ago. Today, reintroductions are on with also some escapes from farms in the Southwest of the country.

So, without going much further, a conclusion could be that contamination of wild boars by domestic ruminants presents a low probability of occurrence, just like the contamination of domestic ruminants by wild boars (Elbers *et al*. 2001). The question of viral transmission from domestic pig to wild boar may be different, specifically in outdoor farming situations. Wild boar farms are to be seen as places where animal really receptive and susceptible to the virus are bred.

Wild exotic suidae in zoological gardens are more to be seen and managed as exotic bovidae (*cf infra*). Under laboratory conditions, peccaries (collared peccary), which belong to the tayasuidae family, so distinct from suidae family, seem to be susceptible to FMD.

6 – Camelidae (Mammals Artiodactyla)
South American camelidae (llamas, alpacas, guanacos and vicunas) like Old World species (two-humped camel and dromedary) are susceptible to FMD but their epidemiological importance seems to be really low, without being totally ruled out. For instance, not so long ago, an FMD outbreak involving cattle, sheep, goats and camels has been described from Mongolia in 2000 (OIE Mai 2000).
Although camelidae chew their cud, they are not classified within Ruminantia by within Tylopoda suborder. In the International Animal health Code, OIE, 2005 edition, they are however included with ruminants for the purpose of FMD chapter (2.2.10, article 2.2.10.1).
Nearly all these species may be seen in Europe, especially llamas, alpacas and dromedary, all considered as domestic animals and being bred in a few farms.

In the case of South American camelidae, it is possible to say that llamas and alpacas are susceptible if infected in the laboratory, but that they do not seem to have any natural importance in the local outbreaks (Lubroth *et al*. 1990, Fondevila *et al*. 1995, Fondevila *et al*. 1996). It is even possible that animals showing clinical signs are unable to transmit the disease to any other animal (Fondevila *et al*. 1995). In this last work, out of 30 animals exposed to the contact of pigs, which had been inoculated, only 3 made the disease. The experiment was performed with 6 pigs, organized in three groups of 2 animals, each group inoculated with a different FMD virus strain (A, O and C respectively). The llamas were put into contact with the pigs, 10 llamas per group of 2 pigs, in distinct and isolated cabinets. Only in a single cabinet (the one with O strain) two animals presented light lesions associated with a seroconversion and a third animal of the same group was discovered seropositive without lesion. In the other two groups and on the other seven animals of the first group, nothing was

noticed. A calf was associated to each group. It became infected and made a clinical disease in every case, like the pigs. In experimental conditions as in Lubroth *et al*. (1990) an inoculated llama was able to contaminate 3 pigs out of 3 put at its contact, an other inoculated llama did contaminated (light clinical form) a single llama in contact out of two and an inoculated cattle contaminated (clear lesions) one out of two llamas in contact.
The transmission risk of FMD virus through embryo transfer in llamas seems to be really lows: 3^{-08} (Sutmoller and Taylor, manuscript, no date).

In the case of the two and one-humped camels, literature is quite contradictory (Richard 1975, Leforban *et al*. 1996, Schneegans 2001). A recent review presents FMD in camels (Wernery and Kaaden 2004). Animals appear however as being susceptible but most of the time, clinical signs are very mild. It seems possible for the one-humped camel as for the two-humped species to transmit the virus to cattle when, in the opposite way, under laboratory condition, virus transmission looks difficult or even impossible. However, being able to transmit the virus means that it has been acquired from somewhere, as these species are not biological carrier or reservoir of the virus.

So, here too, considering these two groups of species, it is clear that their epidemiological importance is not a major one but it is difficult to pretend that there is absolutely no risk. A good management of their numbers, by breeding them away from any other domestic stocks, could be a positive way to protect them. Documented situations in which they really had an epidemiological importance in FMD outbreaks are very few. In South America, the FMD contingency plans only concern ruminants and pigs (Cancino Valenzuela 1988).

7 – Cervidae (Mammals Artiodactyla)
The 2001 FMD epidemic was another occasion to ask about susceptibility of deer to this virus as European populations of red deer (*Cervus elaphus*) and of roe deer (*Capreolus capreolus*) have seen their figures and geographical distributions really increasing these past years, just like wild boar on the continent (Table I). It must be mentioned that precise estimations of their populations are difficult to get. The only solid figures are those of hunting bags, which have also been increasing since the first year when data were collected (in France, 1973-1974 season, figures coming from French hunting agency ONCFS).

Estimations of population figures are just made for red and roe deer and the confidence intervals, not mentioned, are certainly very wide. In France, red deer population could have grown from 38,600 in 1985 to about 117,800 in 2000 and their could be now between 1.5 et 2 millions of roe deer in the country (ONCFS).

	1975	**1980**	**1985**	**1990**	**1995**	**1999**	**2003***
Red deer	6,573	8,824	9,358	13,001	18,592	32,349	49,844
Roe deer	58,563	62,487	98,445	156,948	285,319	395,657	461,689
Wild boar	45,333	57,218	88,413	104,875	211,586	343,628	442,466

Table I : Evolution of hunting bags for red deer, roe deer and wild boar in France. The year mentioned means that of the closing of the hunting season. * Last season published: 2002-2003 (from ONCFS).

The review by Fletcher (2004) points to the famous FMD epidemic on white-tailed deer (*Odocoileus virginianus*) described in 1924 from California, USA, and regularly reported. It could indeed have been caused by an adenovirus.

Species	Population estimations	
	1970 years	***1990 years***
Red deer	190,000	360,000
Sika deer	1,000	11,500
Fallow deer	50,000	100,000
Roe deer	200,000	500,000
Chinese barking deer	5,000	40,000

Table II : estimation of the trends of British deer populations evolution over thirty years (Fletcher 2004)

In France, many blood samplings were realised during capture sessions of red deer, roe deer and wild boars by the National hunting agency (ONCFS). These animals were used to repopulate different regions where their density was then low. These blood samples were made by Afssa Nancy and tested by Afssa Alfort and have always been negative and have not been published.

Under laboratory conditions there is a publication, which presents experimental viral (C type) contaminations in the tongue of all 5 species of deer present in the UK, and transmission between them as well as with cattle and sheep as contacts (Gibbs *et al*. 1975). Sika deer (*Cervus nippon*), fallow deer (*Dama dama*) and Chinese barking deer (*Muntiacus reevesi*) have been introduced in the British islands. Concerning receptivity, roe and barking deer are clearly quite susceptible to the diseases with lethal forms, sika deer shows a less severe clinical disease and the two other species (red and fallow deer) just exhibit a sub-clinical disease. Red deer but mainly sika and fallow deer could become carriers over 28 days. Viral excretion is similar with what is seen in domestic ruminants and under these experimental conditions the virus passed from one species to the others. However, the authors of the paper mention the fact that the conditions of the experiments (close cabinets, high density, small spaces) are not natural conditions. So, the epidemiological importance of deer may be less important than that of domestic ruminants.
The serological survey realised in The Netherlands after the 2001 epidemic on 140 free-ranging wild red deer gave 140 negative results (Elbers *et al*. 2003). In the same time not a single positive case has been notified in the UK on any of the 5 deer species when the growth of the local populations during the second half of the XXth century had also been real there (Table II).

The conclusion could be that contamination of wild deer by domestic ruminants represents a low probability of occurrence and that the contamination of domestic ruminants by wild deer seems nearly impossible. However, deer farms are to be managed like ruminants farms i.e. breeding species whose receptivity and susceptibility is real but rather lower than those of domestic ruminants.

8 – Bovidae (Mammals Artiodactyla)
This is the zoological family to which domestic ruminants are belonging, so it is clear that even if all species have not been tested in laboratory conditions, it is possible to suppose that all are receptive and susceptible and that some of them may become carriers even if we do not know which ones, nor for what types of viruses. In our countries they are found in zoological gardens, with a few American bison (*Bison bison*), water buffaloes (*Bubalus bubalus*) or even yak (*Bos grunniens*) bred in farms. The detail of all the research realised in the field, in Africa or in Asia, as well as the analysis of the outbreaks described in zoological gardens is very rich but can be partly ignore here (Urbain *et al.* 1938, Hedger *et al*. 1972, Pinto and Hedger 1978, Shimshony *et al*. 1986, Hedger 1981, Fowler 1986). African buffalo seems to be live-carrier of SAT FMD virus types and different antelope species can be infected to its contact, some with mild clinical disease some with severe and even lethal clinical forms.

In Europe there are a few native wild bovidae like chamois (*Rupicapra rupicapra*), isard or Pyrenean chamois (*R. pyrenaica*), ibex (*Capra ibex* and *C. pyrenaica*) and one introduced, the Asiatic moufflon or wild sheep (*Ovis orientalis*). Today, none of these species ever play any role in any documented FMD outbreak.

9 – Other mammalian species

Here the list may be rather long, but many of the data are most of the time anecdotic.

What to think of the epidemiological importance of the disease described in kangaroos (marsupials), bears (carnivora), some rodents, tapirs (perissodactyla), hyraxes (hyracoida), either under laboratory conditions, either in zoological gardens and often quite a long time ago (Urbain *et al*. 1938, Neugebauer 1976, Kloes and Lang 1976, Hedger 1981, Fowler 1986, Bhattacharya *et al*. 2003) ?

It is absolutely possible that some of these cases are true and real, just like is true the lesion in the foot of a Guinea pig after inoculation of a FMD virus strain on the spot, but it may also be true that all these species have absolutely no epidemiological importance in FMD epidemics. During an outbreak, actions to be taken around infected premises holding domestic stocks are already quite harsh to realise and so important to be done swiftly and efficiently that it is worth not to be disturbed by questions linked to much less important species and topics.

To close this part, it is possible to recall that some old FMD diagnostic methods used inoculation of the suspected materials to rodents (Guinea pigs and baby mice) and that some vaccines have been produced, up until very recently, by multiplying the virus in young rabbits (Joubert and Mackowiak 1968). This does not mean however that these species do have an importance in the natural history of the disease.

Conclusion - Suggestions

Without being exhaustive, this short bibliographical review distinguishes between some anecdotic situations (zoological gardens) and a whole set of other situations, which potentially may prove to become more serious (wild boar, camelidae, cervidae). However, it must also be stressed that laboratory conditions, really important to use and to know, rarely reproduce field conditions. The analysis and the experience of all previous FMD outbreaks in Europe had shown that a good control of domestic stock (pigs and ruminants) is enough to handle and to eradicate the disease.

Is it important to suggest new laboratory studies for a better quantification of the susceptibility, the level of excretion and the possible length of virus carriage, for different virus types and sub-types and for so many species? May be not for all of them.

The following table reproduces the data here presented (Table III).

Zoological groups	**Receptivity**	**Susceptibility**	**Epidemiological importance**
Birds	No	No	No
Insectivora	+/-	+/-	No
Proboscidae	Yes	+/-	No
Suidae	Yes	Yes	Possible
Camelidae	Yes	+/-	+/-
Cervidae	Yes	Yes	Possible
Bovidae	Yes	Yes	Yes
Rodentia	Yes	Yes (laboratory)	No

Table III : Review of what is known on receptivity, sensibility and on the epidemiological importance of the species mentioned in the text. « +/- » means that the known results are contradictory.

Nevertheless, among the possible suggestions it could be proposed to make a census in our countries of all the different estates and properties holding susceptible species, be they zoological gardens, native or exotic wildlife parks, fenced hunting properties, farms growing non classical species, domestic or not, or circuses with animals, as well as the species and their numbers within all these locations. A good information and knowledge concerning all the animals movements linked to these estates and properties is also to be expected. Another proposal could be to suggest not to create any

new holding with such species too close from a classical breeding area, region or centre so that domestic stock and captive wildlife shall not be present in the same time at the same location. This may prove to be useful not only for FMD.

When dealing with free-ranging native wildlife, the question is yet another one, but from past experiences, it could be anticipated that these animals should not become the origin of too many troubles. At the legislation level, it must be realised that wildlife, free ranging or even within fenced hunting properties is not seen as domestic stock within a farm.

The two following tables could synthesise suggestions to propose when facing one of these different situations, in the absence of any outbreak: Table IV and recommended measures in case of an outbreak: Table V.

To end, it is possible to go to Council directive 2003/85/EC of 29 September 2003 *on Community measures for the control of foot-and-mouth disease repealing Directive 85/511/EEC and Decisions 89/531/EEC and 91/665/EEC and amending Directive 92/46/EEC*. Article 2 (a) is written as follows:
« « animal of a susceptible species » means any domestic or wild animal of the suborder *Ruminantia*, *Suina*, and *Tylopoda* of the order A*rtiodactyla* ;
For specific measures, notably in application of Article 1 (2), Article 15 and Article 85 (2), other animals, such as for example of the order *Rodentia* or *Proboscidae*, may be considered susceptible to foot-and-mouth disease in accordance with scientific evidence. »

It may be possible to think that rodents have been included because of research laboratories and elephants because of zoological gardens and circuses.

Article 15 (1 and 2) allows to derogate to official control measures (Article 10) even in case of a FMD outbreak if this, or these, outbreak(s) are confirmed « in a laboratory, zoo, wildlife park, and fenced area or in bodies, institutions or centres approved in accordance with Article 13 (2) of Directive 92/65/EEC and where animals are kept for scientific purposes or purposes related to conservation of species or farm animal genetic resources... ». Commercial farms of wild boars, camelidae or cervidae are thus not concerned by this derogating measure. Could fenced hunting areas be assimilated to such a situation when they represent a commercial activity and not a nature reserve?

A good application and practice of these rules mean a good communication as soon as possible, as well as a sensibilisation of the public and an adapted training scheme for all potential actors of the contingency plans.

Table IV : Proposal of preventive measures around situations or properties holding non domestic or non « classical » species without FMD outbreak. The terms of Council Directive 2003/85/EC are presented for comparison.

Situations	Council Directive 2003/85/EC	Suggestions
Free ranging wildlife	Not mentioned	Figures knowledge
Fenced hunting areas (with susceptible species)	Not mentioned, not considered a farm	Figures and movements knowledge. Checking of the fences and of the quarantine facilities.
Bovinae other than cattle (bisons, buffaloes, yaks) farms	To be managed like a cattle farm	
Cervidae farms	To be managed like a cattle farm	
Camelidae farms	To be managed like a cattle farm	
Suidae farms	To be managed like a pig farm	
Zoological gardens (with susceptible species)	Susceptible species : Ruminantia, Suina, Tylopoda, Rodentia and Proboscidae.	To propose and to apply adapted bio-security rules
Circuses (with susceptible species)	Not mentioned.	To propose and to apply adapted bio-security rules

Table V : Proposals for management measures in case of confirmed FMD outbreak occurring in one of these situations or properties. The terms of Council Directive 2003/85/EC are presented for comparison.

Situations	Council Directive 2003/85/EC	Suggestions
Free ranging wildlife	Not considered as an outbreak, not mentioned.	No specific action suggested. Hunting seems counter indicated as it could result in large movements of animals and humans.
Fenced hunting areas (with susceptible species)	Not mentioned so culling not indicated.	Stamping out. Checking of the fence. Monitoring of nearby farms with susceptible species.
Bovinae other than cattle (bisons, buffaloes, yaks) farms	To be managed like a cattle farm	
Cervidae farms	To be managed like a cattle farm	
Camelidae farms	To be managed like a cattle farm	
Suidae farms	To be managed like a pig farm	
Zoological gardens (with susceptible species)	Susceptible species: Ruminantia, Suina, Tylopoda, Rodentia and Proboscidae. It is possible to derogate to culling.	To close the property, sanitary surveillance, application of biosecurity rules.
Circuses (with susceptible species)	Not mentioned	Sanitary surveillance, biosecurity rules. Possibilities to derogate to culling.

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Are Dromedary Camels Susceptible or Non-Susceptible to Foot-and-Mouth Disease Serotype O

Soren Alexandersen

Danish Institute for Food and Veterinary Research
Department of Virology, Lindholm
DK-4771 Kalvehave, Denmark

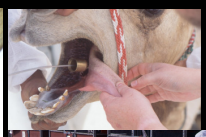
FMD
WIDE HOST RANGE

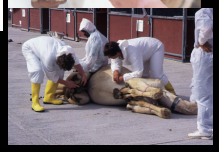
Cattle, sheep, goats and pigs
African & water buffalo

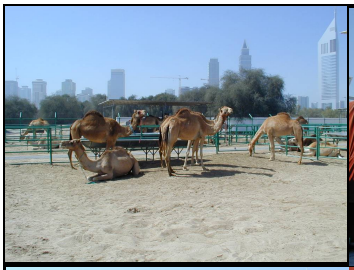
Kudu, impala, warthog, deer

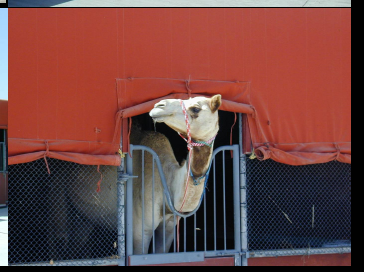
Some other animals, including camels, may possibly be infected

Man extremely seldom, mild and transient







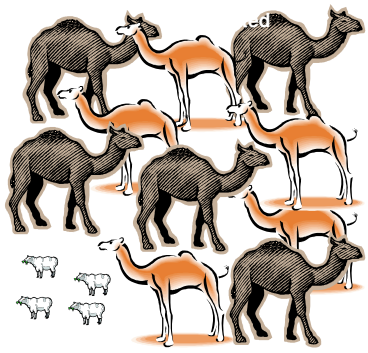


Are Dromedary Camels Susceptible or Non-Susceptible to Foot-and-Mouth Disease Serotype O

Experiment 1

- **Virus**: FMDV O UAE 542-99 (WRL O UAE 7/99) isolated in Dubai from minor epithelial lesions from Arabian gazelles. Used as **5th BHK passage**
- **Animals**: 2 **dromedary camels** and 2 **heifers** inoculated subepidermo-**lingually** with **$10^{7.6}$ $TCID_{50}$**
- **Results**:
  Heifers: **Clinical disease** (relatively mild), **viraemia** and virus detected in swabs and probangs and **development of antibodies**

  Camels: **NO clinical disease**, **NO viraemia, NO** virus detected in swabs and probangs and **NO development of antibodies**
- **Conclusion: no signs of infection in dromedary camels with this inoculum**



Camel Experiment 2

Experiment 2

- **Virus**: FMDV O UAE 542-99 (WRL 7/99) as in experiment 1, but prepared from secondary vesicular epithelium from a heifer in experiment 1, i.e. **used as 1st cattle passage**
- **Animals**: 5 **dromedary camels (3 naive and 2 from experiment 1)** inoculated subepidermo-**lingually** with **$10^{7.8}$ $TCID_{50}$**, 5 naive dromedary camels as direct contacts and 4 sheep as contacts. Also had 2 sheep kept seperately and inoculated in the coronary band as "positive controls".

- **Results**:
  "Positive control sheep" : **Clinical disease** (relatively mild), **viraemia** and **development of antibodies. Typical for FMD in sheep.**
  Contact Camels and contact sheep: **NO** clinical disease, **NO** viraemia, and **NO** development of antibodies
  Inoculated camels: **NO clinical disease**, but **1/3 naive camels had a one day increase in temperature** and **developed a viraemia and subsequently antibodies** to FMDV. The 2 previously exposed camels from Exp. 1 developed antibodies to FMDV

- **Conclusion: 1 out of 3 inoculated naive camels developed a viraemia but did not transmit infection to contact camels or sheep**

Experiment 2 - continued

- **Sequencing of virus**: Sequenced nearly the complete genome (the L-fragment) of **FMDV from the serum of camel 34 at pid 3** (after a single passage in bovine thyroid cells), the inoculum from the heifer and the original 5th BKK inoculum.

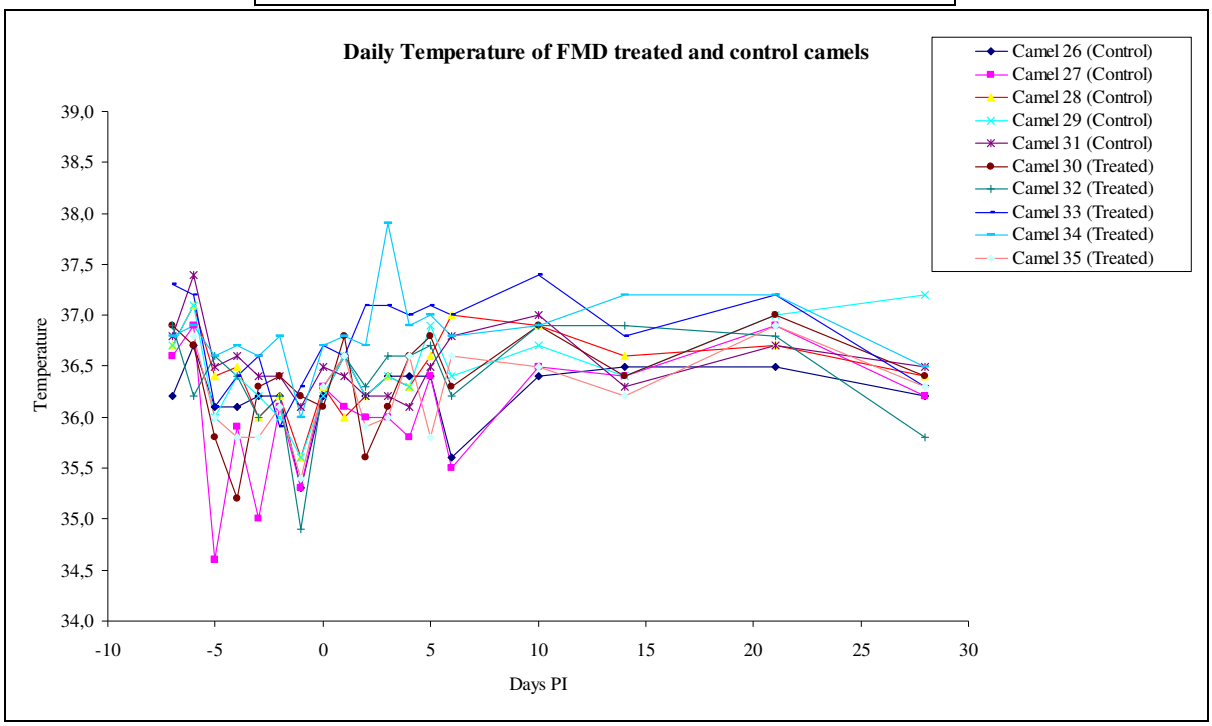
  The virus from the camel is identical to the input virus from the heifer.

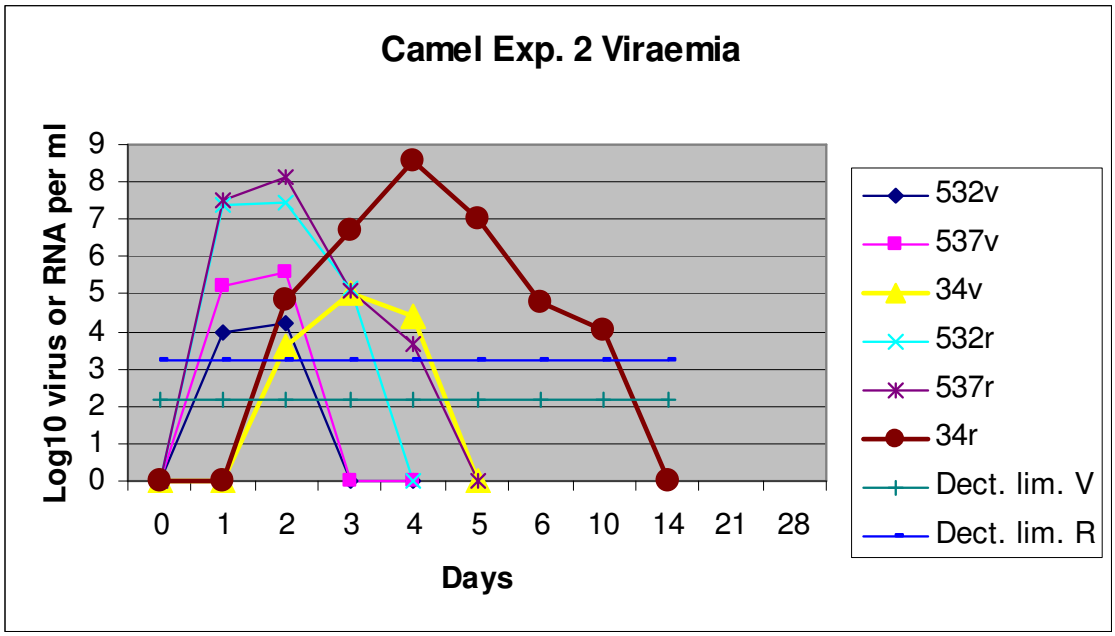
  Interestingly, this virus (from the heifer and from camel 34) is **slightly different from the original inoculum, i.e. the 5th BHK passage**.

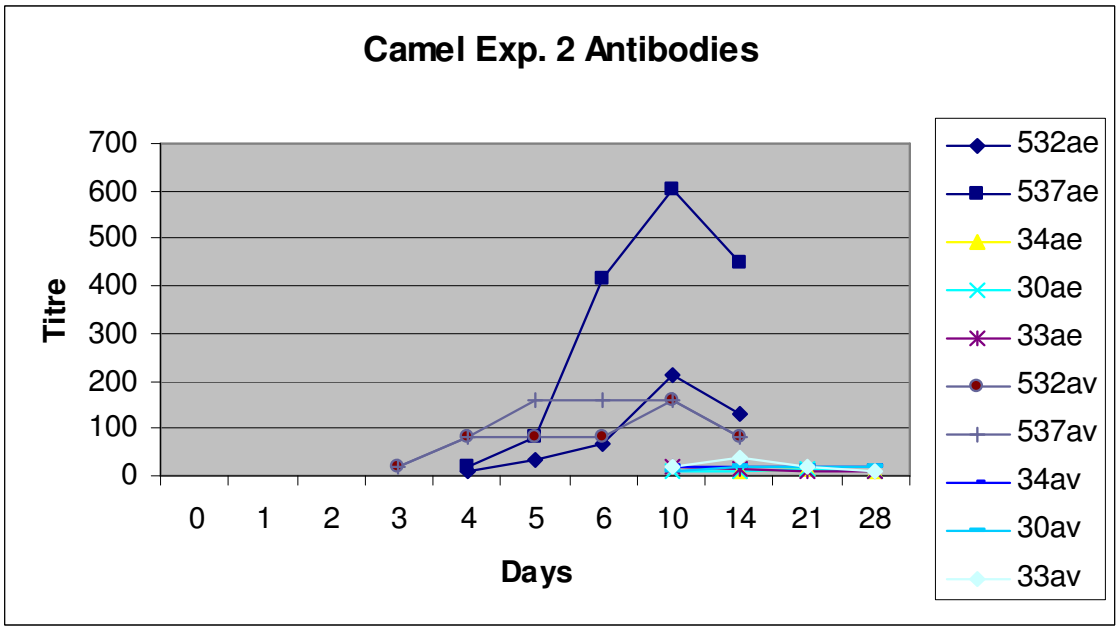
  The original 5th BHK passage had **8 sequence differences** in the L fragment when compared to the two in vivo viruses. Of the 8 differences, 3 were non-coding (2 differences in 2C and 1 difference in 3D).

  Of the **5 coding differences**, 1 is in VP-3 (aa # 158 proline to serine); 2 differences are in VP-1 (aa #13 alanine to threonine and aa # 144 alanine to valine); and 2 differences in 3A at amino acid # 104 (glycine to asparagine) and #129 (alanine to threonine).

  May potentially have to do with BHK cell culture and subsequent in vivo adaptation as the VP-3 change may reflect on heparan sulphate binding, the VP-1 changes (in particular at aa# 144 just before the RGD motif) may change receptor interaction and the 3A changes may also have to do with adaptation to host cells.




Camel Exp. 2 Viraemia
Log10 virus or RNA per ml
0
1
2
3
4
5
6
7
8
9
0
1
2
3
4
5
6
10
14
21
28
Days
532v
537v
34v
532r
537r
34r
Dect. lim. V
Dect. lim. R


Camel Exp. 2 Antibodies
Titre
0
100
200
300
400
500
600
700
0
1
2
3
4
5
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532ae
537ae
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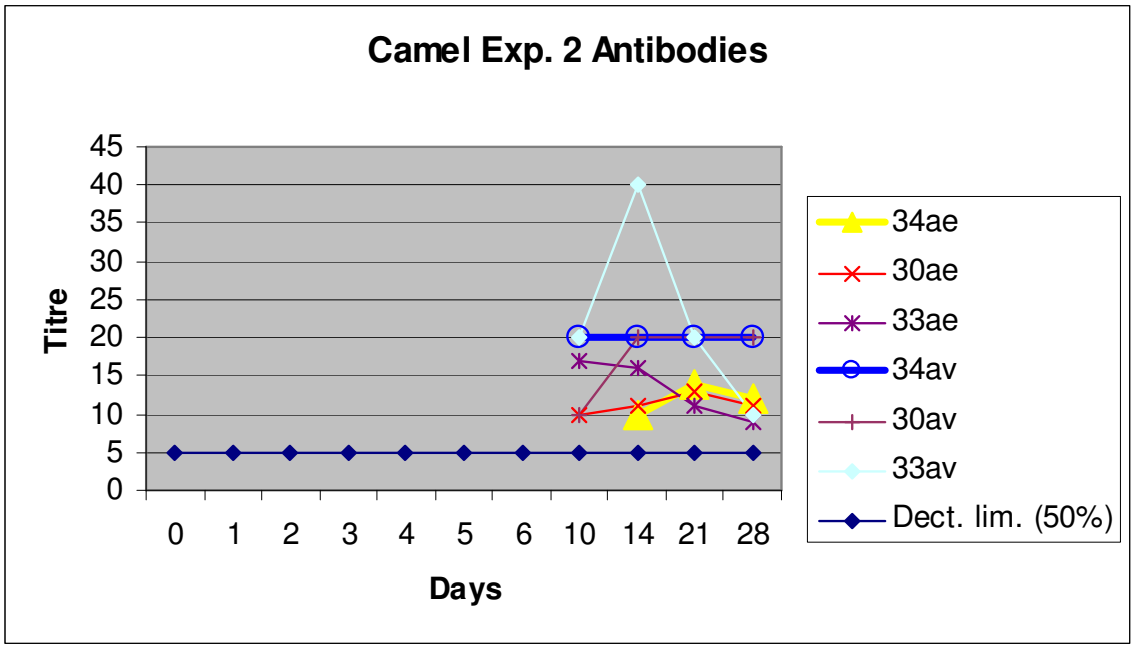

Future experiments

- **As experiment 2 has indicated** that FMDV **serotype O** under certain circumstances infect camels (caused viraemia, antibodies and elevated body temperature in 1 out of 3 camels) when using a fully virulent serotype O isolate, it may also be worthwhile to continue these experiments to get better statistical data.

  Next use 10 naive camels directly inoculated with the heifer 144 type O inoculum - have no contact camels as experiment 2 indicated that contact spread is of **no** significance.

- **Continue the experiments using FMDV serotype A** as this is the other serotype that has been found in the area in or around UAE.
  An isolate from the region (A SAU 22/92 original epi suspension) from infected cattle material has been agreed upon among Ulli Wernery, Soren Alexandersen and Nigel Ferris/David Paton at the FMD-WRL in Pirbright.

- Clearly, the preliminary results from experiment 2 suggest that there is **much more experimental work to** do in order to conclude on the relative, but **low, susceptibility of dromedary camels** to infection with FMDV.

THE END

Thanks to **Ulli Wernery, Renate Wernery, Peter Nagy, Jutka Juhasz, Anita Varga and Winni Schiele** at CVRL, Dubai, **colleagues and staff at Pirbright Laboratory and at DFVF-Lindholm**, and **Gitte Alexandersen, Barup, Denmark**

Research supported by DFVF as well as by **HH General Sheikh Mohammed Bin Rashid Al Maktoum**

SUMMARY

Experiment 1

5th BHK passage FMDV O UAE 7/99 inoculum

2 **dromedary camels** and 2 **heifers** inoculated in the tongue with **$10^{7.6}$ $TCID_{50}$**

Heifers: **Clinical disease** (relatively mild) and virus and antibody detected

Camels: **NO clinical disease**, **NO viraemia, NO** virus detected and **NO development of antibodies**

Conclusion: no signs of infection in dromedary camels with this inoculum

SUMMARY

Experiment 2

FMDV O 7/99 as in experiment 1, but prepared from secondary vesicular epithelium from heifer in experiment 1, i.e. used as 1st cattle passage

5 **dromedary camels (3 naive and 2 from experiment 1)** inoculated in the tongue with **$10^{7.8}$ $TCID_{50}$**, 5 naive dromedary camels and 4 sheep as contacts. 2 inoculated sheep kept seperately ("positive controls" got typical disease, viraemia and development of antibodies).

Contact Camels and contact sheep: **NO** clinical disease, **NO** viraemia, and **NO** development of antibodies

Inoculated camels: NO clinical disease, but 1/3 naive camels had a one day increase in temperature and developed a viraemia and antibodies. The 2 previously exposed camels from Exp. 1 developed antibodies

Original 5th BHK virus had 8 sequence differences in the L fragment compared to the two in vivo viruses. Of 8 differences, 5 were coding differences; 1 in VP-3; 2 in VP-1 and 2 differences in 3A.

Conclusion: 1 out of 3 inoculated naive camels developed a viraemia but did not transmit infection to contact camels or sheep

Appendix 28

Quantification of the transmission of Foot-and-Mouth Disease virus from carriers to susceptible animals: A meta-analysis of transmission experiments

Tenzin [1 a], A. Dekker[b], J.C.M. Vernooij[a], A. Bouma[a], J.A. Stegeman[a]

[1]Department of Livestock, Ministry of Agriculture, District Livestock Sector, Lhuntse, Bhutan.
[a] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL, Utrecht, The Netherlands.
[b] Wageningen University and Research Centre, Central Institute for Animal Disease Control (CIDC-Lelystad), Lelystad, the Netherlands

Carriers of foot-and-mouth disease virus (FMDV) are assumed to be a risk for new outbreaks in susceptible livestock, but this risk has not been quantified. Therefore, a meta-analysis was carried out using data from previously published controlled experiments to quantify the transmission of FMDV from carriers to susceptible animals by estimating the average number on new infections per month caused by one carrier (β), and the decrease in the proportion FMDV carriers in the population.

In total 14 studies were included for the estimation of the transmission rate. Since available data were too limited to estimate the transmission rate parameters between all species, only two categories of susceptible animals were distinguished: 'non-cattle' (buffalo and pigs) and 'cattle'. No relation was found with species being carrier, therefore only one carrier type was distinguished. The transmission parameter β was estimated at 0.1128 (0.000-0.3078) infections per carrier per month for contact with susceptible 'non-cattle', and 0.0076 (0.0000-0.0423) infections per carrier per month for contact with susceptible 'cattle'. Sensitivity analysis showed no big differences when studies were included or excluded.

The decrease of the proportion of carriers was estimated from 6 studies at 0.115 per month.

The estimated parameters are essential for use in risk analysis with regard to the trade of ruminants from infected areas, and for the development of future experiments to further elucidate the role of carriers in transmission of FMDV.

Table 1. List of experiments used for the estimation of transmission rate parameter β.
If buffalo is mentioned, this applies to the African buffalo (*Syncerus caffer*)

Study	No. cases	No. carrier	No. susceptible	N *	Days in contact	Species	FMDV type
Burrows, R (1966)	0	6	4	10	35	cattle to cattle	SAT3
Brooksby, J.B (1968)	0	14	8	22	43	cattle to cattle	A, SAT1
Moonen *et al.*(2004)	0	17	1	18	549	cattle to cattle	A
Bauer *et al* (1977)	0	1	1	2	61	cattle to cattle	A
Bauer *et a*l (1977)	0	2	2	4	274	cattle to cattle	O
Bauer *et al* (1977)	0	2	2	4	274	cattle to cattle	O
Bauer *et al* (1977)	0	2	1	3	274	cattle to cattle	O
McVicar *et al* (1976)	0	1	2	3	42	cattle to cattle	A
McVicar *et al* (1976)	0	1	2	3	42	cattle to cattle	A
McVicar *et al* (1976)	0	1	2	3	42	cattle to cattle	O
McVicar *et al* (1976)	0	1	2	3	28	cattle to cattle	A
McVicar *et al* (1976)	0	1	2	3	28	cattle to cattle	A
McVicar *et al* (1976)	0	1	2	3	28	cattle to cattle	O
Bauer *et a*l (1977)	0	2	1	3	84	sheep to cattle	O
Condy & Hedger (1974)	0	5	6	11	548	buffalo to cattle	SAT1, 2
Anderson *et al (*1979)	0	1	2	3	175	buffalo to cattle	SAT2
Anderson *et al* (1979)	0	1	2	3	152	buffalo to cattle	SAT1
Bengis *et al* (1986)	0	6	6	12	456	buffalo to cattle	SAT1,2,3
Dawe *et al* (1994a)	1	3	4	7	168	buffalo to cattle	SAT2
Hedger & Condy (1985)	0	6	3	9	731	buffalo to cattle	SAT3
Vosloo *et al*. (1996)	2	3	2	5	312	buffalo to cattle	SAT2
Young *et al*. (1972)	0	1	7	8	122	buffalo to buffalo	SAT3
Vosloo *et al*. (1996)	1	3	1	4	198	buffalo to buffalo	SAT2
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller & McVicar (1972)	0	4	6	10	30	cattle to pigs	A
Sutmoller *et al.* (1967)	2	3	6	9	75	cattle to pigs	O
Sutmoller *et al*. (1967)	0	2	6	8	86	cattle to pigs	A
Sutmoller *et al*. (1967)	0	3	4	7	44	cattle to pigs	A
Sutmoller *et al*.(1967)	0	1	4	5	34	cattle to pigs	A
Sutmoller *et al*. (1967)	0	1	4	5	35	cattle to pigs	A
Sutmoller *et al*. (1967)	0	2	4	6	29	cattle to pigs	A
Bauer *et al* (1977)	0	2	4	6	91	cattle to pigs	O

* N: Total number of animals (carriers + susceptible)

Table 2. Experiments with virus isolation from oesophageal-pharyngeal region by probang sampling of cups from carrier cattle following inoculation with FMDV (proportion of cattle positive to FMDV during each sampling period).

Moonen *et al.* 2004		**Salt J.S 1994**		**Kaaden *et al.* 1969**		**Burrows R, 1966**	
dpi *	**proportion positive cattle ****	**dpi**	**proportion positive cattle**	**dpi**	**proportion positive cattle**	**dpi**	**proportion positive cattle**
34	3/17	28	1/1	30	6/8	28	10/10
48	1/17	35	2/5	61	3/5	35	10/10
63	1/17	42	2/3	91	6/8	42	9/10
76	2/17	49	1/3	122	5/8	63	9/10
91	2/17	56	1/3	152	2/4	70	7/10
104	4/17	63	4/5	182	5/8	77	9/10
118	11/16	70	4/5	213	5/6	84	8/10
132	4/16	77	4/5	243	3/7	91	7/10
146	4/16	84	2/5	274	2/7	98	7/10
160	7/16	91	0/2	304	0/7	119	7/10
174	2/16	98	2/5	335	0/7	133	6/10
195	6/16	105	2/5	365	0/7	154	5/10
209	3/16	112	0/5			182	5/10
223	6/16	119	0/2				
237	1/16	126	2/3				
251	5/16	133	2/5				
266	1/16	140	2/5				
287	4/16	147	2/5				
301	4/16	161	2/5				
315	4/16	168	1/2				
329	2/16	175	1/5				
343	1/16	182	0/2				
357	3/16	196	1/4				
385	1/16	210	0/2				
413	3/16						
441	2/16						
469	0/16						
497	0/16						
525	0/16						
553	1/16						
581	0/16						
609	0/16						

* dpi: days post infection
** proportion positive cattle: the total number of cattle positive to FMDV isolation divided by total number of cattle.

Appendix 29

LIST OF PARTICIPANTS

Session of the Research Group
Friedrich-Loeffler-Institute, Greifswald, Insel Riems, Germany
20-23 September 2005

RESEARCH GROUP:

Dr Kris De Clercq (Chairman)
Head
Department of Virology
Section Epizootic Diseases
CODA-CERVA-VAR
Groeselenberg 99
B-1180 Ukkel, **Belgium**
Tel/fax : 32-2-3790400 / 32-2-3790666
e-mail : kris.de.clercq@var.fgov.be

Dr Sinan Aktas
Deputy Director of the FMD Institute
Eskisehir Tolu 7 Kilometre
Ankara, **Turkey**
Tel: 90-312-2873600-201 / Fax: 90-312-2873606
e-mail: sinana@sap.gov.tr

Dr Soren Alexandersen
Research Professor
Danish Institute for Food and Veterinary Research
Department of Virology, Lindholm
DK-4771, Kalvehave, **Denmark**
Tel: 45-72-347833 / Fax: 45-72-347883 direct or 347901
e-mail: sax@dfvf.dk

Dr Emiliana Brocchi
Head
National Reference Laboratory for vesicular diseases
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
Via A. Bianchi, 7/9
25124-Brescia, **Italy**
Tel/fax : 39-30-2290310 / 39-30-2290369
e-mail: ebrocchi@bs.izs.it

Dr Mark Bronsvoort
Lecturer in Epidemiology
Centre for Tropical Veterinary Medicine
Royal (Dick) School of Veterinary Science
University of Edinburgh
Easter Bush Veterinary Centre
Roslin, Midlothian EH25 9RG, **UK**
Tel: 44-774-8804923
e-mail: mark.bronsvoort@ed.ac.uk

Dr Aldo Dekker
Senior Scientist
Laboratory Vesicular Diseases
Central Institute for Animal Disease Control
P.O. Box 2004
8203 AA, **The Netherlands**
Tel/fax : 31-320-238603 / 31-320-238668
e-mail : Aldo.Dekker@wur.nl

Dr Georgi Kirilov Georgiev
Head of Exotic and Emerging Diseases
National Diagnostic and Research Veterinary Medical Institute
1606 Sofia, **Bulgaria**
Tel: 359-2-9383011 / 359-2-9521277
e-mail: georgivet@yahoo.com

Dr Matthias Greiner
Research Professor, Head
Danish Institute for Food and Veterinary Research
International EpiLap and Animal Health Section
Mørkhøj Bygade 19
DK-2860 Søborg, **Denmark**
Tel/fax: 45-72-347108 / 45-72-347001
e-mail: mgr@dfvf.dk

Dr Bernd Haas
Head of National FMD Reference Laboratory
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Boddenblick 5 a
17493 Greifswald-Insel Riems
Germany
Tel/fax : 49-(0)383-51/7-0 / 49-(0)383-51/7-226
e-mail : bernd.haas@fli.bund.de

Dr François Moutou
Responsable de l'Unité d'Epidémiologie Générale
AFSSA - Alfort
22 rue Pierre Curie BP 67
94703 Maisons-Alfort Cedex
France
Tel/fax: 33-149-771300 / 33-143-689762
e-mail: f.moutou@alfort.afssa.fr

Dr Dónal Sammin
Senior Research Officer
Department of Agriculture and Food
Central Veterinary Research Laboratory
Abbotstown, Castleknock
Dublin, **Ireland**
Tel: 353-1-6072721 / Fax: 353-1-6072663
e-mail: donal.sammin@agriculture.gov.ie

Dr Hagai Yadin
Head of Virology Division and FMD Laboratory
Kimron Veterinary Institute
c/o Ministry of Agriculture
P.O. Box 12, Beit-Dagan 50250
Israel
Tel/fax : 972-3-9681619 / 972-3-9681788
e-mail : hagaiy@moag.gov.il

FAO WRL

Dr David Paton (*ex-officio*)
Pirbright Laboratory
Institute for Animal Health
Ash Road
Pirbright, Surrey GU24 0NF, **UK**
Tel: 44-1483-231012 / Fax: 44-1483-232621
e-mail : david.paton@bbsrc.ac.uk

OBSERVERS

EC

Dr Alf-Eckbert Füssel
Head of Sector
DG-SANCO – E2
European Commission
DG Health and Consumer Protection
Rue Froissart 101 – 3/64
1049 Brussels, **Belgium**
Tel/fax: 32-2-2950870 / 32-2-2953144
e-mail: alf-eckbert.fuessel@cec.eu.int

OIE

World Organization for Animal Health
12, rue de Prony
75017 Paris, **France**
Tel: 33-1-44151888 / Fax: 33-1-42670987
e-mail: oie@oie.int

OTHERS

Dr Carsten Pötzsch
Friedrich Loeffler Institute
Federal Research Institute for Animal Health
Seestr. 55, 16868 Wusterhausen
Germany
Tel: 49-33979-80158 / Fax: 49-33979-80200
e-mail: carsten.poetzsch@fli.bund.de

Dr Francis Geiger
Central Asia FMD Surveillance Centre Project
Iran Veterinary Organization
Iran Ministry of Agriculture
Tehran, **Iran**
Tel: 98-912-2360424
e-mail: francis.geiger@yahoo.fr

Dr Maghsoud Jamdar
Director, Veterinary Diagnostic Centre
Veterinary Drugs and Biological Quality Control Laboratories
Iran Veterinary Organization
Tehran, **Iran**
Tel: 98-21-4194501 / Fax: 98-21-44196963
e-mail: mjamdar@hotmail.com

Dr Manzoor Hussein
Regional Epidemiologist
GTFS/INT/907/ITA
c/o FAO Pakistan
UN House, Saudi Pak Tower, 5th floor
Jinnah Avenue (Bule Area)
Islamabad, **Pakistan**
e-mail: manzoorh@isb.paknet.com.pk

FAO

Dr Erika Carlsson
Programme Officer, FAO Tajikistan
UN FAO
39 Aini Street
Dushanbe, **Tajikistan**
Tel: 992 372 210680 / Fax: 992 372 510021
e-mail: erika.carlsson@fao.org

EUFMD SECRETARIAT

Animal Health Service
Animal Production and Health Division
FAO – Viale delle Terme di Caracalla
00100 Rome, Italy
Fax no: 0039-065705-5749

Dr Keith Sumption
Secretary, EUFMD
Tel: 39-065705-5528
e-mail: keith.sumption@fao.org

Dr Tom Murray
Associate Professional Officer, EUFMD
Tel: 39-065705-5124
tom.murray@fao.org

Ms Egiziana Fragiotta
Administrative Clerk
Tel: 39-065705-2637
egiziana.fragiotta@fao.org