Food and Agriculture Organization of the United Nations

european commission for the control of foot-and-mouth disease

OIE/FAO Foot-and-Mouth Disease Reference Laboratories Network

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Foot-and-Mouth Disease
Reference Laboratories
Network

Report of the 5th Annual Meeting of the Network of OIE/FAO Reference Laboratories for Foot-and-mouth Disease, 23-27 November 2009, Delhi

Participants

OIE/FAO Reference Laboratories/Centres

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Belinda Blignaut, Rahana Dwarka	FAO/OIE FMD Reference Laboratory, Transboundary Animal Diseases Programme, ARC-Onderstepoort Veterinary Institute, South Africa
Samia Metwally, Hernando Duque	FAO FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center, Greenport, USA
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Kris de Clercq	OIE Collaborating Centre for Validation, Quality Assessment and Quality Control of Diagnostic Assays and Vaccine Testing for Vesicular Diseases in Europe, CODA-CERVA-VAR, Belgium

Observers from other reference laboratories

Shang Youjun and Lu Zengjun	National FMD Laboratory, Lanzhou Veterinary Laboratory, Gansu, China
Bernd Haas	Friedrich Loeffler Institute, Riems, Germany
Sabenzia Wekesa	Foot-and-mouth disease Laboratory, Embakasi, Kenya

Agenda overview

Day 1: Open meeting with additional scientific colleagues from India

Day 2: Network group meeting discussions on FMD virus circulation and vaccine needs

Day 3: Network group meeting discussions on FMD vaccine matching

Day 4: Network group meeting discussions on test harmonisation

Principal Conclusions

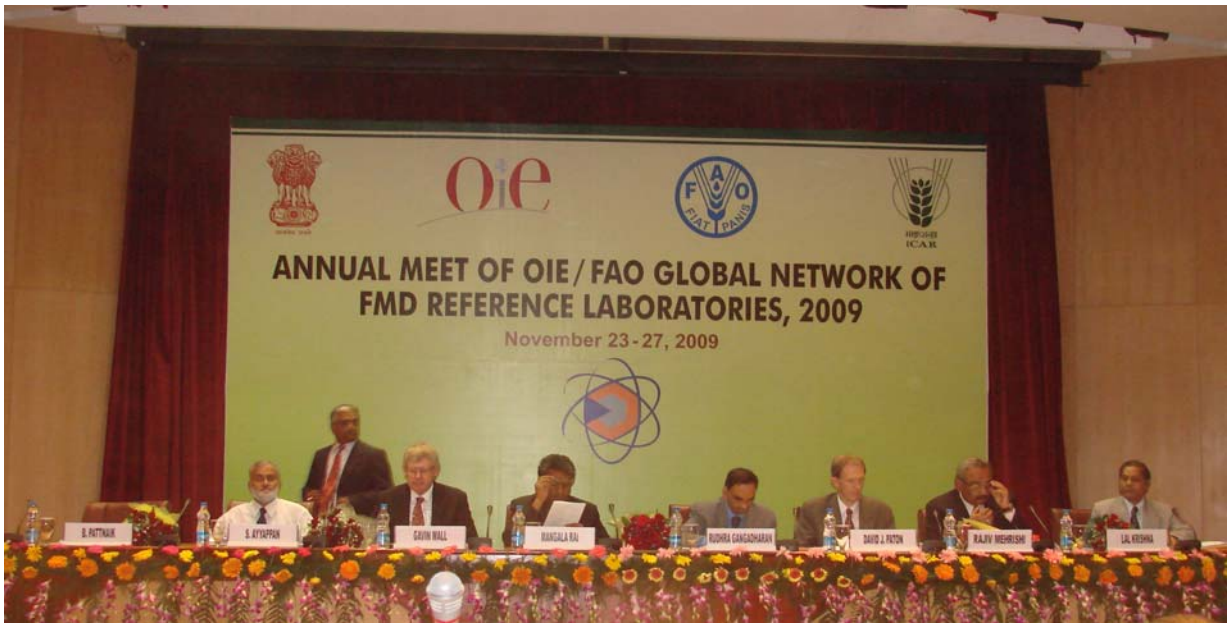
1. The members of the Network are very active individually, but should seek to increase cooperative actions between meetings.
2. The PD-FMD, India is carrying out a wide range of reference laboratory activities at an advanced level and with considerable resources. It already coordinates the surveillance work of a local network of government laboratories in India and should be encouraged to seek OIE reference Laboratory status and to take a lead in establishing a regional network for southern Asia.
3. Provision of proficiency testing schemes is essential to maintain quality assurance and also builds bridges between participants. However, it is becoming an exceedingly demanding and resource intensive job. Hence there is a need to come up with viable models of how to do it.
4. Vaccine matching ring trial work has been hampered by difficulties with arranging documentation and shipment of materials.
5. A decision tree should be developed to explain the procedure for following up and interpreting laboratory data on antigenic changes in field isolates.
6. The recent focus of the network on vaccine matching is justifiable, but reference laboratories should also give due emphasis to ensuring that vaccines used in their regions are of adequate potency and encourage and promote effective delivery.
7. For the naming of the regional virus pools, it was agreed to use the terms “Southern Africa”, “Eastern Africa”, “Western Africa”, “Southern Asia” and “Eastern Asia” rather than “Africa South”, etc.
8. The Network will evaluate the convenience of using the format “SAT2-KEN-2010-1-wrl” for the designation of samples.
9. The Network should prepare lists to show the availability of vaccines and vaccine matching methods and results or data.
10. The network agreed on working towards production and sharing of reagents for vaccine matching testing and to collaborate on enhancing test capability among reference laboratories.
11. It would be useful to elect a vice-chairperson to assist in the management of the Network and its meetings.
12. A gap remains in representation from Western Africa.

Actions

1. The Secretary of the Network will prepare a report of the meeting within one week and circulate it for comment. It will include copies of presentations in pdf file form as an annex. Each reference laboratory should return the report to the Secretary, with their corrections marked in track change mode within one week thereafter.
2. The Secretary will circulate a list of Network participants and their contact details within one week. Each reference laboratory should return the list to the Secretary, with their corrections marked in track change mode within one week thereafter.
3. If not already done so, all reference laboratories will send a draft contribution for the Annual Network report to David Paton by December 18th to allow him to collate and circulate back a report by January 15th. Further comments to be fed back to David Paton by January 29th so that the final report can be submitted to OIE and FAO by February 12th.
4. Panels of materials for the vaccine matching ring trial to be sent to the three remaining laboratories that have not received these. Other laboratories should report their final findings before the end of December. All laboratories are to supply full methodologies and raw test data (including retrospectively for the 2008 ring trial).
5. A teleconference will be organised at the end of 2009 or the beginning of 2010, to discuss the annual report and the results of the inter-laboratory vaccine matching exercise. Other teleconferences should be organised strategically, throughout the year or in response to need.
6. The PD-FMD, India will supply WRLFMD with a selection of viruses and VP1 sequences representing the main genetic lineages circulating in India in the last five years. The PD-FMD and WRLFMD will analyse antigenic and genetic findings jointly in comparison with their own data.
7. The PD-FMD, India will supply their new DIVA test kit to other Network laboratories willing to assist in validation of the method.
8. The Embakazi laboratory should refer serotype C positive field samples to another laboratory for comparative structural protein serology.
9. OVI and WRLFMD to discuss integration of sample data.
10. Network partners to inform the Secretary as to whether or not they would be interested in uploading data directly into ReLaIS, so that a decision can be taken on the value of developing this capability. OVI and WRLFMD to confer on way forward.
11. Dr Kim to report to OIE on the difficulties experienced in shipping ring trial samples, so that the matter can be raised in discussions with IATA.
12. Each reference laboratory should maintain a list of national reference laboratories in their region and this should be shared between network laboratories and made available on websites.

13. Network laboratories should consider arranging a rotation system to enable them to take turns in providing PTS panels to one another on a rotational basis.
14. Each reference laboratory should select a unique 3 letter code for use in naming samples received by them.
15. A list of laboratory codes should be made available on the WRLFMD and ReLaIS websites and be updated as new laboratories are designated.
16. Samia Metwally agreed to prepare a spreadsheet to collate Network information on vaccine matching tests performed and training and reagent needs, as a start to prioritising capability improvement. This work needs to be coordinated with Yanmin Li who is assembling Network protocols starting from information provided through the ring trials on vaccine matching.
17. As far as is possible, given confidentiality constraints, Hernando Duque agreed to prepare a listing of vaccine strains and their manufacturers.
18. An advisory board should be constituted for future ring trials within the Network.
19. Efforts to develop capacity within and representation from Western Africa should be pursued by OIE, FAO and the Network.
20. Secretariat to investigate tele and audio conferencing options and input invited from Network.

Open Meeting



Dr S Ayyappan, Deputy Director General, ICAR, opened the meeting with a brief address expressing his pleasure that the meeting was taking place in Delhi, India and describing the progress that had been made in recent years towards the control of foot-and-mouth disease (FMD) in India. He said that serotype C of the FMD virus (FMDV) has not been detected in India since 1995 and that the introduction of widespread large-scale vaccination has greatly

reduced the number of reported cases of the disease. However, problems remain including the need to increase the scale of vaccination, improve the thermostability of the vaccines and keep the cost of vaccination affordable. He said that for FMD work, a new high security laboratory is being built at Bhubaneswar.

Dr Gavin Wall, FAO Regional Representative for India, emphasised the growing importance of food security associated with an ever increasing human population coupled to higher life-style expectations. Livestock farming had a crucial role in meeting this challenge and support for this sector within developing countries was also a key contributor to poverty alleviation. FMD was one of several important diseases that threatened the livestock sector and its ability to supply our growing needs. At the world food summit on food security, the international community is committed to invest more in agriculture and to eradicate poverty and hunger. Eradication of hunger is complicated and requires science and technology and partnership. OIE and FAO have an agreement in control of transboundary diseases. FMD control in endemic countries will require active partnership to overcome limitation of insufficient epidemiological information, and limited capacity and resources to apply control measures. To that effect, the FAO and OIE action aims to address information gaps needed to develop effective national and regional strategies and to assist in emergency responses. FAO and OIE have a long term roadmap for FMD control in west Eurasia and Southeast Asia, and are willing to partner with India in developing a similar roadmap. Many challenges are present to execute such a roadmap, including but not limited to the cost of vaccine, short duration of immunity, multiple serotypes of the virus and large number of livestock and wildlife.

Dr Yong Joo Kim, Scientific and Technical Department, World Animal Health Organisation (OIE), reminded participants of the conclusions of a recent international conference in Paraguay that had been organised jointly by FAO and OIE to launch a new initiative for global control of FMD. He emphasised the important role played by OIE's International Reference Laboratories as well as the opportunities for financial support to national reference laboratories through a programme of twinning between them and established international laboratories. Dr Kim told delegates that a global scientific conference on FMD was being planned for 2012.

Dr David Paton, FMD Reference Laboratory Network Secretary, gave an overview of the inception, aims, working principles and achievements of the OIE/FAO FMD Reference Laboratory Network to date. The work of the Network had been successful in building contact and trust between members contributing to a fuller and clearer understanding of the threats posed by FMDV globally and to spread of best practice in laboratory methods. The expected outputs from the current meeting would be (1) An update on the global and regional FMD situation during 2009; (2) Regional FMD vaccine strain recommendations; (3) Conclusions on inter-laboratory vaccine matching studies conducted in 2009; (4) Plans for future inter-laboratory vaccine matching studies; (5) An update and draft report on regional

quality assurance undertaken in support of FMD laboratory testing; (6) Agreement on timeframes and actions to develop the Annual Network report for 2009; (7) An action list for the Network during 2010; (8) A better understanding of FMD control in India. He also mentioned that the last meeting of all reference laboratories was in 2006 and that the next will be held in 2010.

Mr Shri Rudhra Gangadharan, Secretary, Department of Animal Husbandry and Food, explained to the meeting the importance of Asia and India for livestock production and especially milk production. Following on the successful eradication of rinderpest and contagious bovine pleuropneumonia, the Government of India had decided to make FMD control its next priority. He described the development of the National Programme of FMD mass vaccination, from its inception in 2003/4 until the present, including plans to expand the area covered in a stepwise fashion until the whole country was included to achieve the goal of controlling FMD in 2020.

Dr Mangala Rai, Director General ICAR, emphasized the need of controlling FMD globally. He further informed the meeting that ICAR has all the capabilities for surveillance and control of the disease in the South Asia Region. He described the latest progress towards the establishment of an international centre for FMD to be built in the city of Bhubaneswar by 2012 that will cater to the SAARC countries.

Dr Lal Krishna, Animal Husbandry Commissioner, said that annual losses due to FMD in India had been estimated at 4.4. billion US dollars and that trade losses were even greater. He pointed out that as well as the traditional livestock species, India was also home to some rarer animals that also suffered FMD, including mithun and yak. He described the status of the current vaccination control programme in which approximately 30 million cattle and buffalo from 54 states had been vaccinated every 6 months for 8 rounds of vaccination. In addition to the vaccination under FMD control programme (FMD-CP) in some selected states, vaccination is also carried out under other programmes (ASCAD and RKVY) in the country. He explained the work and coordination of India's network of laboratories in monitoring the status of the disease and selecting appropriate vaccine strains. He described how the latest figures show a substantial fall in laboratory confirmed outbreaks due to effective surveillance and having the FMD vaccine programme in place. Several states now have a very low FMD incidence and this may enable the development of free zones. Dr Lal Krishna said that serotype O remained predominant, but that the distribution of the other two indigenous serotypes varied temporally and geographically.

Dr Keith Sumption, Secretary, European Commission for the Control of FMD, described the conceptual basis and principal components of the Progressive Control Pathway (PCP) for FMD. He described the different stages envisaged for moving from a situation where FMD was sustained in an unmonitored form to that where the dynamics of disease maintenance were understood, through to planning and implementation of disease control measures. He emphasised the role played by laboratory reference centres in providing services, assisting

in risk management, assessing threats, training and introducing improved/harmonised methods and he updated the meeting on the designation of FAO FMD reference centres. He considered that there was a gap to be filled in the coordination of southern Asian reference centres and that this role could be played by the Indian National Reference Laboratory. He noted the need for regional "animators" to drive forward initiatives and ensure that agreements were fully actioned. He also noted the overlap between threats and solutions in different regional centres necessitating regular contact between them.

Dr Bernd Haas, Friedrich Loeffler Institute, Riems, gave an overview of his institute and the impressive refurbishment work underway with a planned completion in a year's time. He then described a series of experiments designed to study the cross-protection afforded by some serotype A FMDV vaccines in cattle. These studies had shown that high potency vaccines could indeed provide protection against serologically poorly related FMDV of the same serotype so long as very strong antibody responses were elicited although a few animals from the lowest dose group showed protection from challenge without antibody titre. An account was given of a new European project, "Disconvac", that supports a range of research activities related to facilitating vaccine based control measures. The partnership includes laboratories from India, China and South America, as well as from Europe. There is a workpackage that seeks to investigate the benefits of combining vaccine strains of a given serotype to improve the breadth of antigenic coverage.

Ms Anna Ludi, Institute for Animal Health, Pirbright, described her work using a new technique known as antigenic cartography to map the serological relationship between FMDV within a given serotype; in this case, serotype A. The method has a number of potential advantages over the current system of unidirectional matching between a vaccine and a particular field isolate. Firstly, it allows many viruses to be compared to one another and secondly, it should be more robust and reproducible as well as being less dependent upon the supply of vaccine viruses and their antisera, which may be constrained by commercial sensitivities. A fuller evaluation of the method and its potential for linking datasets between laboratories is eagerly awaited.

Ms Belinda Blignaut, Ondestepoort Veterinary Institute, spoke about work being done in South Africa to better understand the viral determinants of antigenic relatedness and cross-protection. Studies of FMDV of the Southern Africa Territory (SAT) serotypes 1 and 2 had shown that it was hard to find vaccine viruses that provided a broad spectrum of antigenic match to field isolates. By studying the amino acid sequences of the capsids of different SAT viruses it was hoped to relate specific changes to alterations in antigenicity. Using a reverse genetics approach it would be possible to be able to directly test the importance of different epitopes. Initial sequencing studies have shown that there are differences in the distribution of amino acid variability between SAT 1 and 2 viruses.

Dr David Paton gave an overview of the global FMD situation and an update of the work of the FMD World Reference Laboratory (WRLFMD) at Pirbright. During 2009, a high number of

samples were submitted due to continued circulation of FMDV serotypes O and A in the Middle East as well as sample submission from parts of Africa and Southern Asia. In South America, confirmed virus activity had been confined to the Northern Andean region. Sample submission to any reference laboratory of the Network did not occur from all countries with endemic FMD. An overview was given of the different serotype distributions and of the way that information from the WRL is provided through quarterly reports available on the WRLFMD website. Some examples were given of where serological matching had suggested potential for problems with the antigenic cover provided by existing vaccines. An explanation was provided of the vaccine recommendations that WRLFMD publish. In addition, there was an update on other activities related to FMD including the development of penside tests and research towards better vaccines. Efforts continue to coordinate research work with other laboratories through the Global FMD Research Alliance (GFRA). A new high security laboratory is being built for the Institute for Animal Health at Pirbright and is expected in 2012.

Network Group Meeting

Regional Discussions

The participants split into groups to discuss current threats and vaccine priorities according to regional virus pools and under the following headings: (1) significant epidemiological events; (2) antigenic changes noted; (3) serological vaccine matching tests done; (4) priority vaccines; (5) gaps in submission; (6) gaps in vaccine selection; (7) threats for 2010.

Pool 1. Eastern Asia. A small number of cases of serotype A and Asia 1 have been detected in China this year. The Asia 1 viruses appear to be matched by the current vaccine used in China, whilst the suitability of vaccines to cover the serotype A viruses is still under study. In South East Asian (SEA) countries, serotypes O and A have predominated with no reports of

Asia 1. Whereas all serotype A viruses in SEA appear to be derived from the same lineage that has evolved within the region for many years, the situation for serotype O is more complex with a mixture of indigenous and introduced viruses. Samples have been analysed at Pakchong from Myanmar, Laos, Vietnam, Cambodia and Thailand. The vaccines used in the different countries are as follows: (1) China, O China 99, A China F/72, Asia 1 China JSWX052; (2) Vietnam, O Manisa and O 3039, A May 97 and A22 Iraq, Asia 1 Shamir; (3) Malaysia, O Manisa and O 3039, A May 97, Asia 1 Shamir; (4) Thailand, O 189/87 (Manisa-like), A 118/87, Asia 1/85 (Asia 1 Shamir-like). There were considered to be no major gaps in submission, although the number of samples analysed is quite small and there have been no submissions this year to Pakchong from Malaysia. In 2010, the main concern in China is over possible continuation of outbreaks caused by serotype A, whilst outbreaks of both serotype O and A are expected to recur in South East Asia.

Pool 2. Southern Asia. In India, outbreaks of serotype O have predominated. About 10% of the ~334 isolates made thus far have undergone testing for vaccine matching to O IND R2/75 and 95% have shown a good match. Until this year, the PanAsia 2 strain was the main one, but in 2009, the "India 2001" strain has predominated. PanAsia2 has been associated with significant disease in wildlife during 2005-08. Most of the 26 serotype A isolates have shown a match to A IND 40/00 and all of the 16 Asia 1 field isolates matched to Asia 1 63/72. These A and Asia 1 virus lineages seem to be unique to India. The predominant strain of serotype A is genotype VII which has an amino acid deletion in an antigenic site of VP3. This virus is distinct from the A Iran 05 strain found throughout the Middle East. The priority vaccine strains are those stipulated in India, namely O IND R2/75, A IND 40/00 and Asia 1 IND 63/72. The meeting agreed on the importance of making a full antigenic and genetic comparison between representative Indian strains and those held in the WRLFMD database.

Pool 3. Eurasia. The main epidemiological events have been the continued spread and circulation of serotype O and A viruses belonging to O PanAsia 2 and A Iran 05 strains. It seems probable that antigenic changes may have conferred an advantage for the spread of the A Iran 05 strain, but this is less clear for O PanAsia 2 which mostly still matches O Manisa vaccine. An isolated case of Asia 1 virus was reported in Bahrain caused by an isolate with closest match to earlier Indian sequences held in the WRLFMD database. An outbreak in 2009, fatally affecting gazelle in the United Arab Emirates, was caused by viruses of the O India 2001" strain. Recent isolates sent to WRLFMD from Pakistan show continued evolutionary change, with some A and a single Asia 1 viruses showing a poor match to A Turkey 06 (A Iran 05 strain) and Asia 1 Shamir, respectively. Elsewhere in the region, Egypt continues to submit samples containing unique viruses of serotype O (related to the O Sharquia 72 vaccine strain) and type A (of the A Africa topotype closely related to that introduced into the country in 2006). In all, about 35 isolates have been matched to a variety of vaccine strains as well as 10 O PanAsia 2 viruses isolated in 2008. The priority vaccines are O Manisa (or similar strains), A Iran 05 strain and Asia 1 Shamir (or similar strains). The major gaps in submission are considered to be from some central Asian

Republics, the Caucasus and some Middle East countries concerned about the impact of transparency on trade. The main problems for vaccine selection are an inability to compare vaccine matching results between centres due to the use of different vaccine strains, unstandardised methods and field isolates that are not shared. Based on previous experience, it may be expected that serotype O but not A will be sustained within the region. An Asia 1 epidemic may be due, since cases are occurring in Pakistan, whilst countries like Iran and Turkey will by now have a low population immunity to this serotype (last seen in 2004). Plans to increase imports of live cattle and small ruminants into the Middle East from Africa and through new trade routes may increase the risk of African strains being introduced. It was felt that the discrepancy between the vaccine strain recommendations from the WRLFMD and those made on a regional basis by the Network caused confusion and it needed to be clearer that the former were intended primarily as a guide for vaccine bank managers in free countries and only included vaccine strains available to WRLFMD and that met the standards of the European Pharmacopoeia.

Pool 4. Western Africa. There is very little available information. In 2008, viruses submitted from Nigeria to WRLFMD were of serotypes O and SAT 2 and revealed a link to other African regions such as Sudan. In 2009, 31 samples were submitted to PIADC-FADDL. Epithelial samples had been collected from an outbreak affecting mainly cattle that had lasted from October 2008 to February 2009. Twenty one of 31 samples were positive and typed as serotype A. Phylogenetic analysis of the P1 and VP1 regions revealed close identity to A Kenya 1984 virus. Collectively, these isolates are more linked to the European and South American old isolates rather than to those of Asiatic lineage of Thailand, Iraq, Iran, Pakistan and India.

Pool 5. Eastern Africa. Samples from Kenya have been typed as SAT 1, SAT 2, O and A, with SAT 1 predominating. The samples were also referred to WRLFMD who have also received samples from some neighbouring countries. Within the region, there are gaps in laboratory capacity and both in technical capability and ability to interpret results. There is a need to develop a strategic plan and network for the region. It would be helpful to identify a specialist from each country who could act as a local animator to encourage action on FMD control initiatives.

Pool 6. Southern Africa. In Southern Africa, SAT1 isolates from 2001-2006 belonged to topotypes I, II and III with outbreaks characterised in Mozambique during 2001-2002, Zimbabwe in 2003, Zambia during 2005 and 2006 and Botswana in 2006. The SAT2 isolates were from Botswana 2002 and 2006; Zimbabwe during 2000/2001-2003, and recently from Nambia and Malawi during 2008 and 2009. Recently, SAT 1 had infected domestic cattle in the buffer zone (Makoko diptank and Phameni diptank). They had heard of outbreaks in Zimbabwe but no confirmation had been received so far.

In Botswana, after 20 years with no outbreaks, a series of 9 outbreaks have occurred since 2002. These have been mostly of SAT 2 and attributed either to incursions from

neighbouring countries or due to contact between domestic cattle and wildlife. The 2008 outbreaks appeared to have been exacerbated by antigenic mismatch between the field virus and the vaccine strains. To try to address the problems associated with lower vaccine matches, efforts are being made to increase antigen payload, introduce new vaccine strains and improve the surveillance of buffalo. The priority vaccines are SAT 2 and SAT 1, but there is insufficient information to be more precise about the strains that should be included. For SAT 1, past vaccine matching results have indicated that vaccine strains are relevant. For SAT 2, recent outbreaks have shown some differences. However, viruses with good correlation to vaccine caused outbreaks recently as well.

Pool 7. South America. There are multiple zones (regarding OIE FMD status) in different countries of the region and maps need to be carefully drawn in order to accurately illustrate the situation. Most of the countries are now FMD-free with or without vaccination. Nine samples were received at PANAFTOSA from Ecuador (n=6) and Columbia (n=3), whilst 19 samples from Ecuador were sent to the Argentinian OIE Reference Laboratory. All of the samples were of serotype O and are of strains indigenous to the region. Venezuela has reported during 2008-2009 a total of 60 FMD outbreaks, (25 serotype O virus and 35 serotype A virus) but has not submitted samples to an OIE reference Laboratory. Nationally, both serotypes O and A have been confirmed. The vaccines used in the region are all single oil emulsions. O Campos and A24 Cruzeiro are used throughout the region, whilst C_3 Indaial is included in Bolivia, Brazil and Paraguay. The justification has been the Amazonas outbreak in 2004. In Argentina, a tetravalent vaccine is used incorporating A ARG 2001 in addition to O Campos and A24 Cruzeiro and C3 Indaial. The role of the OIE reference Laboratories in advising on methodology and standards for vaccine control is considered extremely important. Cattle up to 2 years old are vaccinated every 6 months and thereafter annually, aiming for 100% coverage. For vaccine matching, r_1 values of 0.25 or greater are considered acceptable and the 2009 type O viruses gave values over this threshold when tested at two OIE Reference Laboratories. In some cases r1 values from Ecuador strains were slightly over threshold. Considering the importance of vaccine potency, expectancy of protection is also used to gauge antigenic match rather than relying on r_1 values alone.

In general discussion, the following questions were raised: (1) is it wise to continue vaccination for serotype C? Do we know who is still making serotype C vaccine in different parts of the world? In Kenya, serotype C vaccines are also continuing to be held as some type C seropositive reactors have been found. However, it was questioned whether these could be the result of cross-reaction from infection or vaccination with other serotypes. (2) Do we need a system to raise an alert when reference laboratories obtain information suggesting poor vaccine matching? Considering the poor reliability of the method and the fact that no laboratories have access to all available vaccines, the interpretation of individual results must be handled with care and a decision tree might usefully be developed.

The Reference Laboratory Information System and Sharing of Data

David Paton gave a presentation to illustrate the features of the Reference Laboratory Information System (ReLaIS), the web based information tool set up for the Network, along with the WRLFMD's own website that is managed by Nick Knowles. He demonstrated how to access the prototype sequences that represent all of the main genetic lineages within the seven serotypes. Also, a brief tour was given of how to find the quarterly WRLFMD reports, the annual Network reports and the detailed results including phylogenies and vaccine matching findings from WRLFMD. There are also many other useful pieces of information such as related to contingency planning, sample collection and submission, availability of products and testing services, ageing of lesions, etc. Using ReLaIS, it is also possible to do searches of WRLFMD sample collection as well as to carry out a basic on-line sequence comparison between any new VP1 sequence and those of the prototype strains. The ReLaIS website obtains information on sample submissions to WRLFMD and subsequent test results by direct download from the WRLFMD laboratory information management system. However, it would be possible to devise a system to enable other reference laboratories to enter data electronically and David Paton asked the Network participants for a view as to whether this would be worth developing – i.e. would they use it?

Many of the participants do use these websites on a regular basis and have found them to be useful. Belinda Blignaut informed the meeting that a project has been running at OVI to pull together reference laboratory data on SAT serotype isolates from Southern Africa and it would be useful to discuss whether this information could be integrated with that of ReLaIS. It was agreed that OVI and WRLFMD would lead a working group to investigate further website development needs.

Research and Development for FMD Vaccines in India

Dr Venkataramanan, IVRI Bangalore, described how vaccine production will need to be stepped up in India to meet the needs of the expanding control zones. By 2012, this will encompass 120 million cattle and buffalo. It is estimated that by 2015, there will be a need for 500 million doses per annum. At present there are 5 functional vaccine plants in India with a combined output for the home market of around 150 million trivalent doses annually. Some additional capacity is used for export of vaccines. There are two new plants run by the private sector companies, Brilliant and Biovet respectively. The same three strains (O, A, Asia 1) are used throughout the country, mostly oil adjuvanted. Research on new vaccines at IVRI includes work to express capsids in *E. coli*, yeast, insect cells and plants. There has also been investigation of DNA vaccines and nanoparticles. Other producers are also engaged in vaccine development work, particularly Indian Immunologicals in Hyderabad.

Quality Assurance of FMD Vaccines in India

Dr Srinivasan, Indian Immunologicals (IIL), described the procedures for vaccine preparation and control used in his plant at Hyderabad. The plant produces 750 million monovalent doses per annum. Other producers are: (1) IVRI – 30 m monovalent doses; (2) Intervet – 120 m monovalent doses; (3) Brilliant 30 m monovalent doses; (4) Biovet - 300 m monovalent doses. He described the quality control of raw materials, in process control testing, seed virus tests, tests for inactivation, purification and concentration and final safety and sterility tests. The cost of the final product is controlled by the Government of India at 7 rupees per trivalent dose (less than 0.20 US$). All batches produced are tested by challenge, but this is done by the Manufacturer and not independently. Sourcing seronegative animals for tests is difficult and IIL obtains young calves from herds that have not had outbreaks in the last two years and then grows them up at their own premises. At least six months is needed to introduce a new vaccine strain into production. Critical criteria for acceptance include match to field strains, sufficient mass yield and adequate potency at moderate mass dose.

The Collaborative Vaccine Matching Trial

Yanmin Li gave an overview of last year's vaccine matching trial which had involved distribution of an A22 vaccine strain along with five bovine anti-A22 antisera and five field isolates. Guinea-pig and rabbit antisera were also distributed for ELISA testing. Eight laboratories were invited to participate. Some laboratories achieved reproducible results but others did not. Using the five antisera in a pool gave very similar results to the mean of using them individually and was considered the best approach. Full methodologies and raw test data were not received from all participants. For 2009, it was decided to extend the study by supplying additional viruses to be matched and by using the pooled BVS only. Twelve laboratories were invited to participate and eleven agreed to do so. All were encouraged to carry out the tests using their own methodology as well as that supplied by WRLFMD. Great difficulties were experienced with the processing of documents and the sending of samples and this greatly delayed the progress of the work. More documents including various certificates and contracts than previous years were required from WRL for some countries to obtain the import permits from their authority. Also, some airlines which were available in the past had refused to take on board biological infectious materials or shipments containing dry ice since the beginning of this year. Three laboratories had still not received the panels. Results had only been received from about half the participants by the start of the meeting. A preliminary analysis suggested that as last year, there were both similar and disparate results from different laboratories. A fuller analysis would be carried out once more data is received. Participating laboratories were encouraged to ensure that they send their methodologies and the raw data of their test results including titre values as well as calculated r values.

Yanmin Li also reviewed the vaccine matching work done at WRLFMD between 2000-2009 during which time ~ 685 isolates had been matched to a variety of vaccine strains (serotypes "O" > "A" > "Asia 1" > "SAT2">"SAT1">"C"). There was a lack of antisera against some

representative antigenic variants, especially for SAT viruses. Unless manufacturers provided WRLFMD with vaccine strains and their antisera, it was not possible to do matching work with them or make recommendations for their use. In the case of a recent serotype Asia 1 virus from Pakistan that did not give a match against the Asia 1 Shamir vaccine strain, capsid sequencing was being used to study the surface exposed amino acid changes likely to be responsible for the altered antigenicity.

Measuring Vaccine Coverage in the Field

Rosanna Allende spoke on this subject from the South American perspective. Historically, vaccine coverage had been deduced from the number of doses sold to farmers, but this had been replaced by serosurveys to measure antibody levels as a measure of population to immunity. Either the virus neutralisation test (VNT) or the liquid phase blocking ELISA (LPBE) were used for this purpose. A correlation study was made between protection to challenge and antibodies titre measured by VN or LPBE. Analysis of data from the correlation study had enabled a cut-off antibody titre to be established for each vaccine strain that accorded with adequate protection and this value ranged from log 2.1 -2.4 in LPBE. Serosurveys were designed to minimise sample size whilst maintaining 95% confidence in results with a 15% maximum sampling error. The design takes account of the correlation between age and likelihood of protection (due to increasing doses of vaccination) and of the laboratory test characteristics (sensitivity and specificity). Sampling is carried out close to the time of revaccination, when population immunity will be lowest. Overall, Dr Allende emphasised that whilst it was necessary to use adequately matched vaccines, the requirement to ensure adequate potency and an effective programme of vaccine administration were at least as important.

There was a discussion on whether a cut-off chosen on the basis of potency tests reflected the level of immunity required to prevent spread of virus rather than clinical protection. Also whether more focus could be given to young animals that were more difficult to immunise successfully and therefore represent a critical control point. It was mentioned that at this stage, vaccination programmes in South America aim to eliminate clinical cases. Different sero-surveys based on NSP antibody detection are used to check viral circulation in the field.

Repeatability of vaccine matching tests

Alexei Scherbakov described studies in ARRIAH to monitor the repeatability of their vaccine matching work. The laboratory had had difficulties in establishing a trustworthy methodology, despite a visit to receive training at WRLFMD. In 2006, the use of complement fixation testing had been replaced by VNT and LPBE. A set of bovine anti-vaccine antisera were prepared by vaccinating two groups of five cattle with either O Manisa or O Russia 2000 vaccine. Each set of five antisera were pooled and used to carry out vaccine matching between the homologous vaccine strain and a heterologous field isolate of the O PanAsia 2

strain. Tests were repeated ten times over a period of a few months. In most repeats, the r_1 value was indicative of protection. However, in a minority of cases, the results indicated no match, whilst the range of matching values was quite wide. Considering the rarity of new pandemics, it was suggested to use in vivo rather than in vitro cross-protection tests.

Following this presentation, Yanmin Li presented data from WRLFMD on their repeatability studies with VNT. This also revealed variability in the values obtained, but qualitatively, the results were generally repeatable. Work on comparison with LPBE is in progress.

The presentations provoked considerable discussion. It was pointed out that the variability of VNT had been identified many years ago and that was why some workers had advocated a switch to use of LPBE. However, LPBE was quite difficult to establish due to the dependence upon suitable rabbit capture antibodies and the difficulties of standardising the amount of trapped antigen. Furthermore, there was often a discrepancy between the values obtained by VNT and LPBE. Considering the difficulties inherent in the vaccine matching tests, their results need to be interpreted cautiously and must be verified by repetition and testing of multiple isolates. The view was expressed that false negative matching results are more likely than false positive ones, when using the VNT.

Work is needed to identify the sources of variability in the tests. In the case of VNT, changes in cell culture sensitivity, the influence of serum supplements and differences in virus passage histories were considered likely to be important factors.

The South American system whereby expectancy of protection is measured from the seroreactivity of field isolates against a large panel of vaccine antisera without reference to the homologous titre has many advantages. However, it does require larger panels of sera against each vaccine strain and preferably that multiple challenge test data is available in order to calculate protective cut-off levels.

Development and Use of 3AB3-DIVA in India

Dr Mahapatra presented work to develop, validate and utilise an indirect ELISA to detect antibodies to the 3AB3 FMDV non-structural protein (NSP) that had been expressed in *E coli*. The test was a cheaper alternative to commercially available test kits and had been found to have good levels of sensitivity and specificity, even after multiple vaccination of cattle with Indian vaccines. A survey of 32,000 cattle in different regions of India had revealed an overall NSP seroprevalence of 31%, with ranges from 6-46% at a state level. Reanalysing the data after ranking seroreactivity in different strength categories further emphasised the differences between states. Several Network laboratories expressed a willingness to receive the new test kits in order to evaluate its sensitivity using panels of antisera maintained in their archives.

Test Improvement and Harmonisation

Proficiency Test Schemes Organised by WRLFMD

Yanmin Li described the history of proficiency test schemes (PTS) provided by WRLFMD over many years. The number of participating laboratories has risen, whilst both the complexity of the panels supplied and the difficulties in arranging shipments have also increased. It is sometimes difficult to obtain up to date information on who needs to be contacted at which laboratories. Local rules on transport, customs handling and receipt of samples have led to samples being delayed, returned or destroyed. Airlines dislike taking packages containing large quantities of dry ice and some refuse to take on board any such materials. There are regional PTS priorities with regard to both tests and strains of FMDV and this can make it difficult for a one-size fits all approach.

The North American Laboratory Network

Samia Metwally described the proficiency testing organised by her laboratory for the North American Laboratory Network and for laboratories in Canada and Mexico. A "train the trainees" strategy was employed along with an annual proficiency test scheme. The aim was to achieve equivalency in test outputs, regardless of protocols used. FMD tests covered included cell culture, antigen ELISA, RT-PCR, 3ABC ELISA and VNT. Each participating laboratory contributed to the preparation of the sample panels and monthly conference calls were used to get everything ready on time. The approach has been very successful in developing contacts between laboratories and in helping to build up their capacity and capability.

The SADC Workshop on Serological Monitoring of FMD Vaccines

Rahana Dwarka described this workshop aimed at improving vaccine evaluation. It took place in Botswana with participants from Botswana, South Africa, Mozambique, Malawi, Zimbabwe and Swaziland. Amongst the recommendations were the following: (1) to use the WRLFMD LPBE protocol; (2) to produce validate and distribute bovine vaccinal sera for SAT1, 2 and 3; (3) to continue technical support to national reference laboratories for post vaccination monitoring (PVM); (4) to provide validation data for reagents distributed for PVM. Despite the closure of the EU funded SADC project, this initiative will continue. The Botswana reference laboratory is producing the reagents for LPBE, namely bovine vaccinal sera, antigens and control sera.

South American PTS Initiatives

Rosana Allende described the external quality assurance (EQA) scheme operated by PANAFTOSA for the national FMD reference laboratories in each country of South America. The PTS encompasses antigen detection ELISA, NSP serology, RT-PCR and structural protein serology by LPBE. For the serology exercise, each lab is asked to test every sample six times.

Non-conformities are followed up closely to identify problems with the samples or with the procedures of the non-conforming laboratory.

South East Asia PTS Initiatives

Wilai Linchongsubongkoch provided an overview on four SEAFMD laboratory Network meetings. The OIE reference laboratories in Thailand has jointly organised a regional PTS with Australia Animal Health Laboratory. There was also a one-day training course on how to pack and transport dangerous goods. They had 16 laboratories that participated. A test was carried out at the end and laboratories that passed received a certificate. Incoming samples for virological testing had been scored for their quality: good, if >1 g material; fair, if 0.4-1 g; poor, <0.4 g. Of those samples surveyed, only 8% fell into the "good" category, whilst 60% were scored as fair and 32% as poor. A regional PTS attempted in 2008 on LPBE was beset by problems and had no clear conclusions. A new PTS is underway for 2009 using serology and antigen detection, and involves eight South East Asia laboratories, as well as eight more from Thailand itself.

In general discussion, the following points were made: (1) A list of national laboratories from different regions and their contact details should be maintained on a website to encourage update by the laboratories themselves. (2) Network laboratories could benefit from participating in each others' regional PTS, but to avoid excessive additional burden it might be best if a rotation could be agreed according to which a different Network laboratory would supply to the others each year. Alternatively, or as well, WRLFMD could be supplied with panels each time a PTS is carried out. (3) The Network sponsors need to be made aware of the considerable resources required to carry out PTS and the difficulty this presents if there is no or inadequate dedicated funding.

Nomenclature of FMD Viral Isolates

David Paton presented a short talk on this on behalf of Nick Knowles from WRLFMD. The aim should be to choose a system that is as short as possible and at the same time as informative as possible. A computer friendly format is also desired. Including a code for the laboratory of designation is also required to avoid two different viruses from the same country and year being given the same name. There was disagreement on the advisability of including a state or other within country designation of location. This could be optional? After discussion of several formats, the following suggested format was put forward for further consideration: Serotype-Country of origin-year of collection-chronologically numbered sample for that year-laboratory code. The style and ordering should be consistent and the chosen format was: SAT2-KEN-2010-1-wrl.

Break-out Session on Regional Vaccine Selection

The Network members split into smaller groups and then reported back in plenary on their discussions.

Africa plus USA: Tests used for vaccine matching are as follows; Ondestepoort: VNT and LPBE (results correlate); Gabarone: VNT and LPBE (problems with VNT and prefer LPBE); Embakazi: VNT available (but rarely used for r values); Plum Island: VNT, but only starting to use for r values (little experience with LPBE). Overall conclusion is that there is room for improvement in test capability and a need for additional reagents – vaccines and vaccinal antisera. Samia Metwally agreed to develop a spreadsheet to collate Network capability, needs and priorities which differ regionally. This would be followed up with a teleconference to agree actions for training and reagent preparation.

South East Asia, China and Europe: (1) All protocols should be submitted to Yanmin Li at WRLFMD for collation and comparison of methodologies. (2)The r1 values should be carefully interpreted by jointly considering titres and the potency of the vaccine to be used. The test should be at least repeated twice before submission of the results. A set of guidance will be developed for interpretation and follow-up of vaccine matching findings (need for repeats, minimum numbers of isolates examined, acceptable variation between repeats, evaluation of titre values, etc). This will build upon the guidance already found in the OIE Diagnostic Manual. (3) It was agreed that each network laboratory would carry out intra-laboratory repeatability tests using their own materials. (4) Where possible, larger panels of materials for the ring trial should be distributed so as to provide sufficient materials for inter-laboratory repeatability trials as well as the ring trial works and to reduce the need for repeat distributions. (5) An advisory board should be established for the ring trial work. The advisory board should meet early next year to consider the current ring trial results and plan next steps. (7) WRL will provide an antisera list with gaps and to circulate this list to the network partners for them to fill in the gaps if they can. (8) BVS raised from the regional vaccine manufacturer should be made available for public use and also the vaccine virus if possible.

India: Extensive use of vaccine matching using both VNT and LPBE both at Mukteshwar and at local labs. Data can be provided to show that good repeatability and reproducibility is achieved. However, inconsistency between VNT and LPBE is not uncommon. Would be interesting to organise a ring trial using Indian vaccine strains and a MTA to make these available to WRLFMD is under development. A genotype specific RT-PCR for serotype A and Asia1 has been developed to help in diagnosis.

Summary of workgroups agreed

1. An advisory board for planning and overseeing future PTS – to be convened by Yanmin Li
2. A website development working group initially composed of members from WRLFMD and Ondestepoort
3. A reagent and methodology collation and analysis workgroup to be convened by Yanmin Li and Samia Metwally

Secretariat

David Paton invited discussion on the role of the Secretariat and whether there was interest in changing this. Keith Sumption suggested that OIE and FAO would need to be involved in any discussions on this matter. The possibility of establishing a vice-chairperson was agreed to have merit and nominations were invited from Network members.

Next Meeting

There may be opportunity for some of the Network to meet up at the forthcoming international meeting of all OIE reference Laboratories in June, in Paris. However, this date is rather early for the next Network meeting and would rather serve as an opportunity to plan the 2010 meeting[1]. It was agreed to hold this immediately after the Open Meeting of the EuFMD in Vienna, 29th September – 1st October. Since many of the Network members will attend this meeting, it would be logical to follow this with the 2010 network meeting and it was agreed to hold this in Pirbright, 4th – 6th or 7th October 2010.

Feedback from Participants on Value of Meeting

Feedback was sought from each Network member on what they had liked and disliked at the meeting. The following suggestions were noted: (1) more discussions on development and validation of diagnostic assays. (2) subgroup needed on overcoming problems of sample shipment and use of alternatives to sending live virus. (3) more on exchange of diagnostic reagents to improve capacity. (4) a list of vaccines and their producers is needed. (5) ring trials on vaccine matching has been the most useful exercise. (6) a template should be developed to collate all needed information prior to the meeting. (7) more emphasis should be given to vaccine control as well as vaccine matching. (8) more feedback to the secretariat is needed to develop the agenda of the meeting. (9) improved communication is needed between meetings. (10) the closed session of the meeting should not be attended by others. (11) a round table format would be better for the closed meeting than a lecture theatre arrangement. (12) a list of participants and their contact details should be collated, reviewed and displayed on the website. (13) the agenda focuses on practical issues and the use of break out discussion groups and not just set piece speeches is highly beneficial. (14) the openness of discussion was highly appreciated. (15) still some gaps in regional representation – especially Western Africa. (16) would be good to involve wider representation of national laboratories; a web-based discussion forum would be useful. (17) use of short and to-the -point presentations were much appreciated. (18) name tags should be worn. (19) tele or audio conferencing arrangements need to be investigated.

A vote of thanks was extended to Dr Pattnaik and his team and to Dr Paton and Amanda Hewitt at WRLFMD. Concluding remarks were made by Drs M S Oberoi and M V Subbarao from FAO who considered that the Indian laboratory had clearly made enormous progress in

[1] It could also provide an opportunity for an official steering group meeting

developing to International reference Laboratory standard and was in a good position now to coordinate laboratory activities at a regional level. Finally, Dr Lal Krishna closed the meeting, emphasising the importance of the development of the Bhubaneshwar laboratory for India as well as for regional cooperation.