REPORT

EAST AFRICAN REGIONAL FMD LABORATORY NETWORK

MEETING AND WORKSHOP

Kenya, Nairobi

8- 12 February 2010

Executive Summary

A four day meeting and workshop was convened in Kenya, with financial support of FAO and the EuFMD Commission, to share results from national Foot-and-Mouth disease virus (FMDV) risk monitoring programmes including those where sample collection/typing had been supported by FAO/EuFMD, and to identify how to overcome problems of low virus recovery rates in the first phase of support through improved field sampling and outbreak investigation procedures. The workshop also brought together, for the first time, laboratory experts from the region and gave an opportunity to establish a network of laboratory experts on Foot-and-Mouth disease (FMD) that could assist to improve the services available in the region at national and regional level and to provide information and guidance to decision makers on control of FMD. The workshop was organized with a one day symposium in Nairobi and a 3 day field exercise during which outbreaks were investigated, samples collected and where possible, tested immediately in the Regional Veterinary Laboratory.

The participants represented 7 Eastern African countries, FAO and were technically supported by an FMD laboratory diagnostic expert from the National Veterinary Institute, Technical University of Denmark, Lindholm Laboratory in Denmark. The excellent spirit and local organization, the commitment of all to play their part to the full, the sharing of information and expertise and access provided by the Department of Veterinary Services, Kenya, made the entire workshop a very great success. The very positive feedback from the participants and desire to apply new approaches in their situation suggested that the impact of the event, and the network, should be significant for improving FMD monitoring, risk communication and eventually, management.

Recommended actions in 2010

The participants developed a Workplan for 2010 for the Network, and requested FAO to provide support for some of the activities indicated.

1) The Network to be formally established by FAO under East African Regional Laboratory Network (EARLN) for FMD and associated with the Global OIE/FAO FMD Ref lab network;

2) Regular communication achieved between the network participants, assisted by a **website for EA FMD network;**

3) Building up capacity: in 2010at least 3 laboratories in the region should participate in the Annual Proficiency Testing Service (PTS) of the WRL in Pirbright (UK), and during the year the network should identify how PTS could be developed in a manner which is which is both appreciated and feasible for eastern Africa. A further training event should be organized in 2010, to focus on

    - improving efficiency of FMDV typing including alternatives to virus isolation;
    - **practical laboratory workshop**: to be held at the end of 2010, with focus on molecular diagnostics, validation and suitability for Eastern Africa;
    - integrating the new tests into FMDV monitoring, for example feasibility of sampling animals at markets (normal and clinically affected):

4) Training on sampling for field staff: the network webite/resources should include training tools, using those which already exist (videos on the collection of probang samples, swabs, epithelial tissues etc.):

5) the Network should prepare a **draft manual for training** within 3 months (by end of May), alternatively building on the Pirbright one and adapting it for use in the region:

6) further support from FAO is needed to continue with network activities:

7) an **Annual Meeting** is needed, to share information and build regional capacity and mutual recognition of the quality of services. The Meetings could (as per 1st meeting) be linked to training or field work if desired. Participants from Ethiopia indicated their willingness to host the 2011 meeting (Jan 2011).

Acknowledgements

The support of the Director of Veterinary Services, Republic of Kenya, and his staff at laboratory and field levels to enable the field and laboratory work is greatly appreciated. The success of the event is also the result of the excellent work over many weeks of Dr Wekesa and her team in Kenya to set up the field and laboratory support units. The assistance of FAO-Kenya and EuFMD Secretariat staff in Rome to rapidly and efficiently organise logistics, working with Dr Wekesa, was appreciated by all. The participants (the Network) also acknowledged their governments/administrative organizations and for the FAO and EuFMD support to attend the meeting.

REPORT OF THE WORKSHOP

Day 1: FMDV circulation in the Eastern Africa Region: situation and relevance of new tools and approaches to improve FMD monitoring

The meeting was held with participants from 7 Eastern African countries, namely: Kenya, Uganda, Tanzania, Burundi, Somalia, Ethiopia and Sudan . Also in attendance were representatives from FAO offices in Kenya, Somalia and EUFMD.

Opening and Background to the Workshop

The meeting was opened by a representative to the Director of Veterinary Services, Kenya, Dr. Marete. He appreciated the efforts by the planners to have a meeting of this kind and the opportunity given to Kenya to host the meeting. He re-emphasized the fact that FMD is a menace in the region and it is only through concerted efforts and regional approach that its control can be attained. He commended the swift gathering of such scientists, which responded to the regional networking of the OIE/FAO FMD Reference Laboratories Network meeting held in India in November 2009. .
Dr Litamoi (of the Emergency Centre for the control of Transboundary Animal diseases FAO/ECTAD, Kenya) in his presentation entitled "FAO and laboratory networking in Eastern Africa" remarked that the EARLN in existence has been holding such network group meetings before, whose emphasis has been laid on HPAI, Rift Valley Fever and other transboundary animal diseases other than FMD due to budget constraints. However , he advised that the FMD laboratory network should come up with proposals and work plans, should and approach his office for discussions on the way forward. He mentioned that his office could facilitate international conferences, development of SOPs, and procurement of laboratory items among other activities. Dr.Marete observed that the main challenges for veterinary laboratories in the region include staff shortage and continuity in reagents availability.

He reiterated the importance of networking and that the FMD network would be an important part of the EARLN and advised that there is the need for closer links with the epidemiology networks. He further pointed out that the main capacities of networks should include organizing trainings, study tours to share expertise and information. He mentioned the importance of ownership of networks for the regions. FAO role would be to launch, support, and coordinate.

Section 1: FMD virus circulation in eastern Africa in past two years (2008-9)

Presentations were given by Drs. Abraham Sangula and Sabenzia Wekesa.

In his presentation Dr. Sangula gave the following remarks and conclusions:

- type O is the predominant serotype responsible for most outbreaks in livestock. Current outbreak strains in EA are genetically distant from vaccine strains;
- Type A has a high genetic diversity and is widely distributed across the region whereas type C has the least genetic diversity, and its current status needs survey in Kenya were it was last reported in 2004;
- Among the SATs, SAT2 is most widespread. Wildlife role (Buffalo) is important in epidemiology but very few isolates are available and there is therefore a need for sampling. SAT3 activity in Uganda needs survey;
- Overall, the resolution of circulation patterns of FMDV in EA is undermined by limited number of isolates and there is need for constant sampling.

Dr. Wekesa in her presentations also pointed out the following:

- The fact that there is an increased number of outbreaks, some occurring after vaccination with homologous vaccines; the reason could be emergence of new strains, hence the need for vaccine matching. The vaccine strains currently in use date as far back as 1971.
- On the East African Pool 4, she remarked that some East African countries such as Uganda, Rwanda, Burundi and Sudan have been using Kenyan vaccines yet little research has been done on their effectiveness. According to molecular studies done at Pirbright, there is evidence of divergent strains having evolved, hence the need for vaccine matching training and undertaking;
- She also appreciated support by EU FMD for the project on FMD sampling in the Somali Ecosystem in Kenya, whose final report was still being drafted, and requested that the project should be extended.

Section 2: Country presentations

- Each country gave its presentation; from which the circulating serotypes were established. It was evident that capacity for sample collection, submission and diagnosis was lacking in most of them.

Situation analysis:

- Ethiopia: Lab already started, training for lab staff, SOP, quality manual, and quality control system. **Problem**: Lack of proficiency testing.
- Kenya: Lab exists a bio-security level 3, undertakes ELISA and VNT, needs establishment of PCR, training in vaccine matching, QA/QC, SOP, support in reagent acquisition and sample collection.

- Sudan: FMD Lab capacity needs reassessment and to be extended, ELISAs and VNT are currently applied, establishing standard Lab for molecular biology although PCRs (conventional and realtime) exist.
- Uganda has a lab (National Animal Disease Diagnostics and Epidemiology Centre) at BSL 2 and has been performing a number of serological tests including Ceditest®/Priocheck® FMDV NSP and SP-O ELISAs, 3 ABC –ELISA, seven-type serotype specific blocking ELISAs (SPBEs). PCR technology has been set up over the last three years.
- Both Uganda and Tanzania have RT-PCR thermocyclers.
- Tanzania is currently using only antigen detection ELISA to determine the circulating isolates and need to establish the molecular diagnostic techniques. There are efforts to upgrade a new lab to BSL3 so as to facilitate more diagnostic techniques to be carried out.
- Rwanda and Somalia have no FMD lab capacity. Somalia relies on Kenya
- This information was later used to revise and prepare a "Summary report for Eastern African virus pool, that was submitted to the WRL on 11th Feb for inclusion in the OIE/FAO Global Report" (Annex 6).

Section 3: recent advances of relevance to fmd surveillance in the region

- Diagnostic advances –
- The importance of lesion aging for selecting samples, and to identify time-line of transmission events –
- the role and application of participatory epidemiology to better understand why outbreaks of FMD occur :

Section 4: wildlife and FMD risk in eastern Africa

- buffalo populations in eastern Africa ; opportunities for sampling to identify FMD risks :

Section 5: Network - What is needed

In a round-table discussion on what the FMD ref Lab network could achieve, the participants discussed issues affecting networking and identified a wish-list for the network.

1. Proposed approach: appointment of focal points in each country, build database for expertise within the region to promote interaction of international and regional expertise;
2. Who will play the leading role in regional labs? Need for a focal person/ coordinator;
3. Conclusions: Good example of network initiatives: Rinderpest eradication.

DAYS 2-4

Section 6: Field work in Nakuru

The participants transferred to Nakuru, about 140 km from Nairobi in the Rift Valley, on the 8th February . The location had been chosen since FMD outbreaks had occurred in the Province 1-2 months before the workshop, and there is good road access to animal populations in which FMD is frequently found. In addition, the Regional Vet lab is located in Nakuru, allowing processing of some samples (not under high containment therefore only kits with inactivated/safe principles were set up; NSP and type O serology). Veterinary officers on the ground were gave useful details on animal populations and information on trends of FMD in Nakuru district and guide to the farms to be visited. The DVO for Nakuru municipality informed the participants that the municipality serves as a central market for animals from surrounding districts, and hence is highly susceptible to FMD among other diseases. He also remarked on the endemicity of FMD involving different serotypes all year around , therefore the municipality was ideal for that kind of training.

The participants were divided into 3 teams to visit 3 farms simultaneously where each team chose a leader and a reporter:

1. The clinical team, also known as the "dirty team" whose task was to examine suspect animals and ensure that the most appropriate samples were taken and most accurate epidemiological (infected herd data), aiming at finding the earliest lesion (for sampling) and the oldest (for tracing).

Table 1. Types of specimen to be collected and preservative media to be used at different stages of FMD

	Plain	1ml of 0.04M PBS 25 in 2mls	1.5 ml RNA later	1ml of RLT later in 2mls tube	50% PBS 0.04 M +Glycerolin 5ml	5ml of 0.08M PBS M25	Blood in plain vacutainer tube	Penside medium
1. Acute cases (1-4 days)								
Swab FAO (under the tongue)	√	√	X	√	X	X	X	X
Swab Lindholm (over the tongue)	X	√	X	√	X	X	X	X
Probang	X	X	X	√	X	√	X	X
Lesion	X	X	√	X	√	X	X	√
Serum	X	X	X	X	X	X	√	X
2. Subacute and chronic cases								
Swab FAO (under the tongue)	√	√	X	√	X	X	X	X
Swab Lindholm (over the tongue)	X	√	X	√	X	X	X	X
Probang	X	X	X	√	X	√	X	X
Serum	X	X	X	X	X	X	√	X

2 The epidemiology team (Epi team) also called the "clean team" whose task was to try to establish the timelines of events that could have introduced the current infection and which could allow spread. The team leader was an experienced person in participatory epidemiology and introduced and lead the participatory information gathering.

3. Laboratory team (Lab team). This was the support team, mainly consisting of the laboratory persons to receive and test samples and correlate their findings with Epi and clinical teams. This team was led by a Rift Valley Regional Laboratory staff.

The location, history and collected diagnostic specimen collected from the three visited farms A, B and C in the two districts are shown in table 2.

Table 2. Diagnostic specimen collected, clinical disease history and observations made in the farms A, B and C visited during the EA-lab meeting.

	Animal No/Spp	Localty of Farm	History of FMD	Observation	Samples taken	Remarks
1	A1 Adult bovine	District 1	Suffered FMD 2 months ago, few weeks after vaccination	No clinical signs	Mouth * Swabs, Probang and Serum	
2	A2 Adult bovine	District 1				
3	A3 Adult bovine	District 1				
4	B1 Adult bovine	District 1	The animals had been brought to this farm from Dundori district during an outbreak in Dundori, a couple of weeks before		Mouth * Swabs,Saliva Probang and Serum	
5	B2 Adult bovine	District 1				
6	C1 Calf bovine	District 2	Vaccinated on 1st January 2010. About 3 weeks after vaccination and 3 days after visit to cattle dip, the animals started showing clinical signs of FMD	Healing tongue and feet lesions, limping	Mouth* Swabs, Saliva and serum	Estimated duration >7 days.
7	C2 Calf bovine	District 2				
8	C3 Adult bovine	District 2		Feet and teat lesions	Mouth* Swabs,Saliva, Probang and serum	
9	C4 Adult bovine	District 2				

* Mouth swabs indicate both over and under the tongue swabs

MAIN LESSONS LEARNT FROM FIELD INVESTIGATIONS:

1) Team work is paramount;
2) Good coordination leads to success;
3) Need for more training of field staff to sample correctly and collection of epidemiological data (herd data);
4) Need for further investigations on the use of swabs and RNA preservation at site;
5) The cost/difficulty of such intensive investigations need to be taken into consideration ;
6) The wide variety of media with different preservatives are used for different types of collected specimens and stages of the disease;

The Clinical Team presented their findings on the Final day. As the freshest lesion found was >7 days, the penside test for FMD antigen could not be applied.

Section 7: Laboratory report

The lab team received several samples, namely: Sera, Probang, Saliva and Mouth swabs. However, only sera were subjected to the laboratory tests at that time. The rest of the samples were packaged and refrigerated in readiness for transportation to Embakasi and subsequently to WRL Pirbright and Lindholm respectively. The need for international ref labs at this stage for molecular detection methods (PCR/sequencing) presents difficulties for rapid strain typing in Eastern Africa. The assays used locally were 3ABC ELISA (PrioCHECK® FMD NS) and PrioCHECK® FMDV type O and the results are shown in table 3.

For the first time, FMD serology was performed at the local laboratory and provided results within about 12 hours of sample receipt (tests set up in evening of the day collected, and results obtained the next morning) . It was observed that the FMD NS test showed more strong positive result than FMD serotype O kit which indicated that the recent outbreaks might be caused by another serotype (other than serotype O). This, in their opinion, was in agreement with report of lab finding that showed it was Serotype SAT2.

Serum, saliva, mouth swabs and probang samples were packaged and transported to FMD laboratory Embakasi for further analysis and further transport to WRL and Lindholm. Virus neutralisation tests (VNT) were performed at the Embakasi Laboratory and results are shown in table 3.

Table 3. Results for sera analysis by PrioCHECK® **ELISA and VNT where highlighted areas indicate positive titres by VNT**

Specimen identity.	**ABC ELISA****	**VIRUS STRAIN**					
		O		**A**	**C**	**SAT 1**	**SAT 2**
		K77/78	**ELISA****	**K5/80**	**K 267/67**	**T 155/71**	**K52/84**
A1 District 1		+1.51		0.9	0.9	+1.81	0.9
A2 District 1		+1.36		0.9	0.9	+1.96	+1.36
A3 District 1		1.04		0.9	0.9	+1.36	0.9
B1		+2.56		+1.65	0.9	+1.51	+2.86
B2		+2.11		+1.65	0.9	+2.26	0.9
C1 District 2		0.9		0.9	1.04	0.9	1.04
C2 District 2		+1.81		0.9	0.9	1.2	+2.86
C3 District 2		+1.36		1.04	0.9	0.9	+3.01
C4 District 2		+1.51		0.9	0.9	+1.81	+2.86

** Please note, the results were based on visualisation of colour change as the spectrophotometer was not working at the Rift Valley Regional lab.

Conclusions

- NSP and type O serology results must be added;
- there is a different profile to serology results from the animals sampled at the two locations (District 1 and District 2);
- the high VNT titres to types O O and SAT1 at District 1 suggest recent infection with two virus types (or cross-reactions?);
- cross-reactions to type O seem less likely as in District 2, high titres were found to O and SAT2;
- the high VNT tires to SAT2 at District 2suggest SAT2 was responsible, even though the suspicion from the investigations wasSAT1 as the vaccine used on 1st January had a SAT2 component (check);
- if SAT1 or SAT2 are confirmed, this creates a suspicion that the vaccine used is not well matched to the circulated FMDV strain as outbreaks occurred about 3 weeks post-vaccination;
- the value of additional sampling in the area at time to locate and sample early lesions is essential, if virus is to be isolated and vaccine suitability identified.

SECTION 8: Recommended actions for the network in 2010

The participants, on day 4, developed a work plan for 2010 for the Network, and requested FAO to provide support for some of the activities indicated.

1) **Information:**

- The network has to be established and institutionalized (since it is a new finding), so there is the need to lay down a set of rules;
- Need for wildlife FMD information – wildlife surveillance to be planned;
- Establish a **website for EA FMD network/ wiki space**.

Note: There could be an issue of transparency. No one will be compelled to divulge what is not permitted by their country.

2) **Building up capacity:**

- Quality assurance should be undertaken: what is appropriate for FMD labs in the region?
- **Proficiency Testing**: it was observed that Panels from WRL Pirbright are usually paid for, hence Eufmd could fund if the labs specifically request for it. Eufmd could support 3 countries laboratories to participate in panels (Kenya, Ethiopia, Sudan)

Note*:* Some of the panels are not of interest for one specific lab, therefore the appropriateness of panels needs to be considered.

- Suggestion: Panvac vet lab in Ethiopia has good capacity. It is involved in independent vaccine quality testing and could be used for training; but they should also join the proficiency testing.

- Suggestion: Possible further training event in 2010: focus on diagnosis of fmd: swab results, relevance of alternative methods.

- Suggestion: Training, application, and a **practical workshop**: to be held at the end of 2010?

    - with focus on molecular diagnostics, validation and suitability for Eastern Africa,

    - and integrating the new tests into FMDV monitoring, for example feasibility of sampling animals at markets (normal and clinically affected)

- Suggestion: follow on project for submission to FAO could include development of tools, their validation, plus surveillance.

3) Training of field staff

- Field sampling, use of training tools which already exist: videos for probang, swabs.

- The network should come up with a **draft manual for training** within 3 month (by end of May), alternatively building on the Pirbright one and adapting it for use in the region.

Conclusions:

- It was agreed that further support from FAO is needed to continue with network activities.

- an **Annual Meeting** is needed, to share information and build regional capacity and mutual recognition of the quality of services; the Meetings could (as per 1st meeting) be linked to training or field work if desired. Participants from Ethiopia indicated their willingness to host the offers to host the 2011 meeting (Jan 2011)

ANNEX 1: LIST OF PARTICIPANTS

PARTICIPANT	COUNTRY
Chrisostom Ayebazibwe	Uganda
Abraham Sangula	Kenya
Gelagay	Ethiopia
Tesfaye	Eth- Trainer
Habiela	Sudan
Kirsten Tjørnehøj	Lindholm - Denmark
Lazare Butununguu	Burundi
Gafarase	Rwanda
Chanasa Mpelumbe-Ngeleja	Tanzania
Sabenzia Wekesa	Nairobi, Kenya
Joseph Marete	Deputy Director of Vet Services, Nairobi, Kenya
Keter Kenneth	Nairobi, Kenya
Teresia Kenduiywo	Nairobi, Kenya
Eunice Chepkwony	Nairobi, Kenya
William Birgen	Nairobi, Kenya
Joseph Kubugi	Nairobi, Kenya
Bonaya Galgallo	Nairobi, Kenya
Judith Mumo	Nairobi, Kenya
Hellen Mutua	Nairobi, Kenya
Joseph Litamoi	Nairobi, Kenya
Mahmoud Hassan (Jabra)	Somalia
Paul Rwambo	FAO Somalia
Nadia Rumich	EUFMD
Keith Sumption	EUFMD

ANNEX 2

	DAY 1: Monday : Nairobi	*Panafric Hotel*
0830	Registration	
09.00	Opening /Welcome Remarks.	Director of Vet Services – Kenya; FAO Representative
	FAO and Lab networking in Eastern Africa	Dr Joseph Litamoi
09.10	Introduction to Course, organization of the week Progressive Control, Regional Roadmaps for FMD, and lab networks	Dr Keith Sumption
	Part I: FMDV circulation (focus on 2009)	
09.25	Overview of FMD epidemiology in East Africa with focus on Kenya/Uganda	Dr Abraham Sangula
09.45	FMDV surveillance overview for 2009 – Report from Pool 5 (Eastern Africa)	Dr Sabenzia Wekesa
10.00	Discussion	
10.10	Short reports: FMDV events in 2009 and lab findings, (EuFMD supported partners)	10 minute reports
	Kenya	Dr Wekesa
	Ethiopia	Dr Gelagaye, NVRI
10.30	**Coffee break**	
10.45	Short reports: FMDV events in 2009 and lab findings	
	Sudan (EuFMD supported partner)(10 mins)	Dr Habiela
	Tanzania (5 mins)	Dr Chanasa Ngeleja-Mpelumbe
	Uganda(5mins)	Dr Chris Ayebaziibwe
	Burundi (5 mins)	Dr Lazare
	Somalia (5 mins)	Dr Hassan Jabra
11.15	Discussion	
11.20	**Part II:New developments and methods relevant to monitoring FMD control and threat detection**	
11.20	Diagnostic developments: 1) Virus detection/confirmation;2) Sero-monitoring	Dr Kirsten Tjornehoj
11.40	Single Outbreak investigation procedures : selection of tests, and establishing timelines depends on lesion aging	Dr Sumption
12.00	Role of participative epidemiology methods to identify FMD events and epidem patterns	Dr Tesfaye Rufael
12.20	Sampling opportunities – Buffalo and FMD in East Africa	KWS
	Part III: East African FMD network	
12.40	An East African FMD network – what do we want? What is needed? What should be the priorities for actions in the coming year?	Q&A to the Panel (panellists to be decided)
1.00	**Lunch**	
1.30	Group work on East African FMD network: aims, objectives, actions, actors, events	
2.30	Feedback	
3.00	**Close/Tea**	
3.30	**Departure Nakuru.** **Arrival Merica hotel.** Short Briefing/meeting to discuss programme Days 2-4 ; (known/suspected FMD outbreaks)	
	DAY 2: Tuesday	**Start in meeting room of Hotel Merica**
0800	Briefing on the current FMD situation in Nakuru (recent/ongoing/suspected) outbreaks	DVS Staff - Nakuru
	Group work and tasks during field investigation	Dr Sumption, Dr Wekesa, Nadia
	Clinical investigation/sampling team	Identify team, leader and roles
	Epi-investigation team	Identify team, leader and roles
	Lab support (Nakuru) and epi-information team	Local staff from Nairobi/Nakuru
0915	**Leave for field investigation.**	**Packed lunch**
pm	Return and Initial sample processing/test set up (lab); Meet to plan Day 3;	
	DAY 3: Wednesday	
Start **Time to be decided;** Day 3 may be a follow-up outbreak investigation (2nd); or: teams work on their Day 2 reports; Lab team report findings by 1300; Afternoon – clinical and epi- teams present reports. If no outbreak investigation, Any free time can be given to additional offered talks or group work (recommendations for network.		
	DAY 4: Thursday	
Start time	to be decided. If days 2+3 involved OB investigations then Day 4 morning is for compiling reports and presentations	
3.00	*Guided Visit to Nakuru National Park/ Ecology of African buffaloes*	
	Conference Dinner (in the Park)	
	DAY 5: Friday Departure for Nairobi in the morning	