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# GFRA

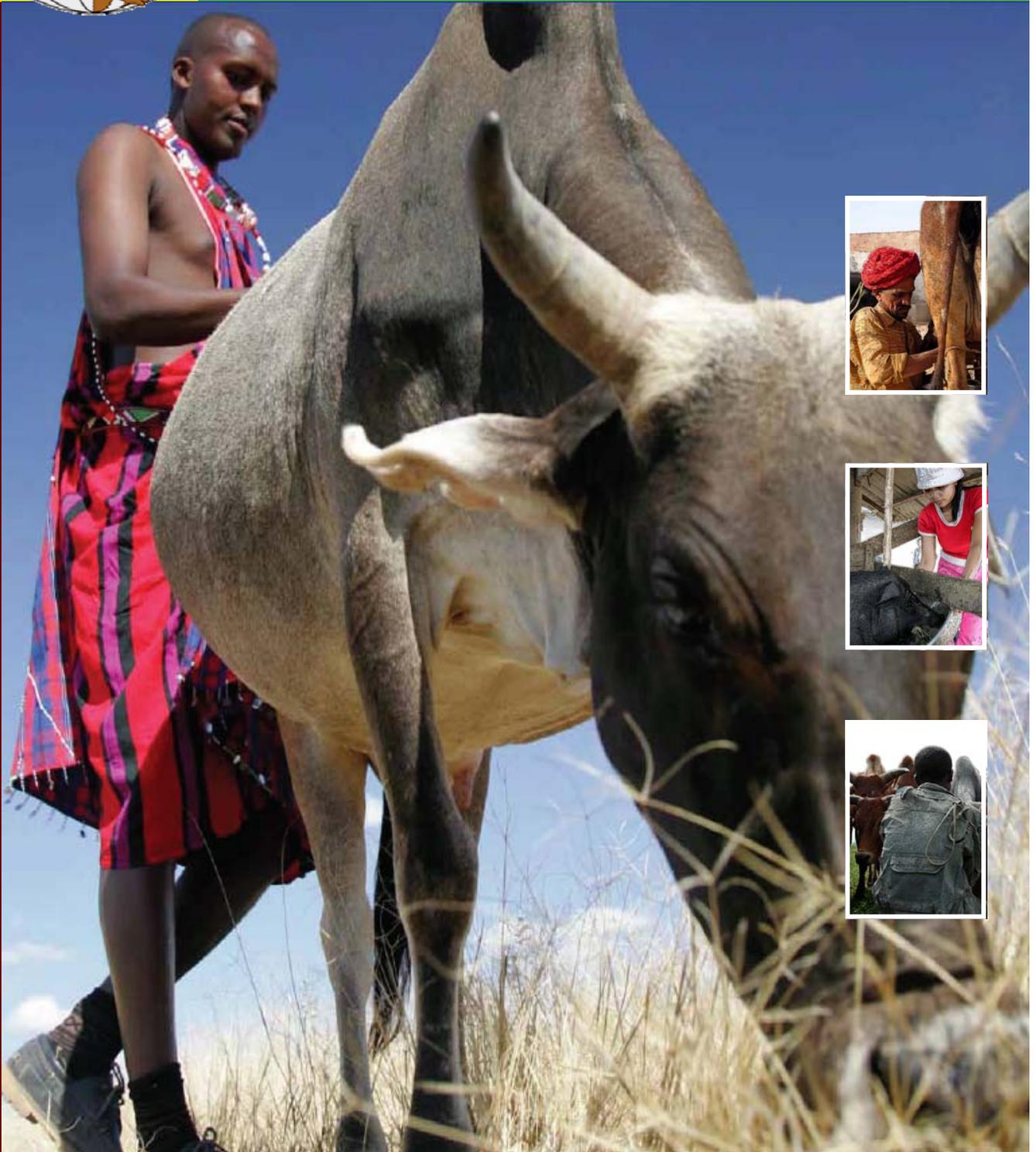
## NEWSLETTER

*Fighting Foot-and-mouth disease*

**together**



Global Foot-and-Mouth Disease Research Alliance (GFRA)



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## Special points of interest:

- *The Netherlands is still spending money on research on FMD emergency vaccine*
- *Foot-and-mouth disease (FMD) is endemic in many countries especially within Africa and southern Asia*
- *Some areas report up to three outbreaks per year often with different serotypes*

This annual vaccination campaign brought down the number of outbreaks very quickly, but due to introductions from Germany and Belgium in the period 1961 to 1966, the positive effect of vaccination was almost gone. In this period the strains that affected the Netherlands transmitted very effectively between pigs. And especially on mixed farms with pigs and cattle outbreaks occurred. Only when Belgium and Germany also implemented an annual vaccination scheme, was FMD brought under control. In the Netherlands prophylactic vaccination was a success story, but was this only due to vaccination or did other factors play a role?

As can be seen from the graph the notification of outbreaks of FMD was already implemented for many years, which indicates that an efficient veterinary service was present. The development of the FMD vaccine had started before the Second World War with optimisation of virus culture using many different *in vitro* techniques, including tissue culture of foetal skin tissue and finally tongue epithelium collected in slaughterhouses. During the Second World War the Waldmann vaccine production system was implemented by the German occupier. In this system virus is cultured by infecting live cattle in the tongue and harvesting the tongue lesions. These tongue lesions are cut into

# Why is FMD post vaccination monitoring necessary?

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As in most infectious diseases control of FMD can be achieved by separation of infected animals and susceptible animals. This barrier can be a physical barrier, or an immune barrier. In 1953 the Netherlands decided as one of the first countries to use annual prophylactic vaccination of cattle as a control tool for FMD. This decision was based on the fact that due to the enormous number of infected farms (approximately 10 000 infected farms in 1950 and 1951) a physical barrier would have too many consequences on the normal agricultural business.

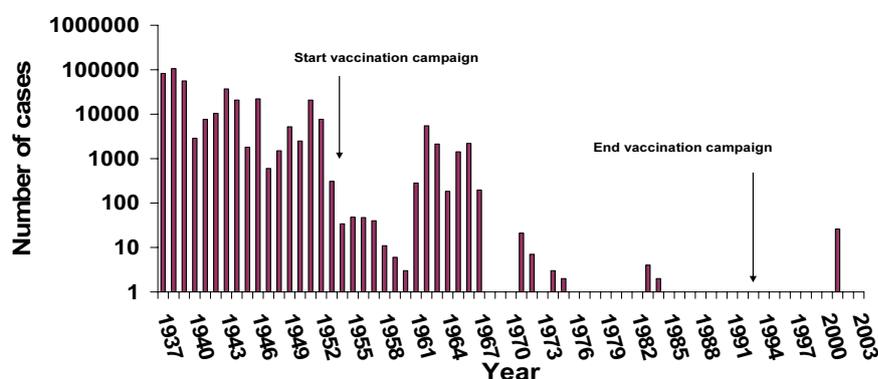


Figure 1: Number of FMD affected farms in the Netherlands between 1937 and 2012. The arrows indicate the start and the end of a nationwide vaccination programme of cattle in the Netherlands.

What can we learn from countries that successfully controlled FMD with vaccination?

small pieces, the virus inactivated with formaldehyde and the virus is absorbed to aluminium hydroxide. Only after the Second World War (1945) could the work of Frenkel on culturing FMD virus on surviving tongue epithelium be continued which made large scale production possible. In the North of the Netherlands there had been already experiments with the experimental vaccine before the nationwide vaccination programme was implemented. The whole history shows that there had been quite an investment in research on FMD vaccine for more than a decade before the nationwide vaccination could be implemented.

Research was not only necessary during development of the vaccine but also to support FMD vaccine Quality Control. There are several publications on various aspects of FMD quality control from Dutch researchers. The reason the money was spent on research lay in the fact that the costs of FMD infection are high. Even at this time The Netherlands is still spending money on research on FMD emergency vaccine. Most of the current research in the Netherlands is focussed on the quantification of transmission of FMD with and without vaccination. Also when FMD was causing outbreaks in the Netherlands in 2001 FMD emergency vaccination was used, as research had shown that it would be able control the outbreak with vaccination.

In recent history there are more countries that use FMD vaccination and spend millions of dollars on acquiring FMD vaccine, but they often do not spend any money on quality control of the vaccine and the vaccination programme. Still they expect to have the same success that has been seen in countries that invested in quality control and research. So what kind of research is essential?

To be able to select a matching vaccine countries should have an efficient monitoring sys-

tem to know which strains are circulating and which vaccine strains should be included in the vaccine. This can partly come from passively acquiring samples from clinical cases. The outcome of the passive surveillance should be checked by taking random serum samples from the population to see if the serological responses match with the serotypes isolated in the passive surveillance.

When it is clear which serotypes and subtypes should be included in the vaccine, the vaccine can be acquired. When the serotype in the vaccine will match very well with the outbreak strains a vaccine that contains three times the dose that would protect 50% of the animals should be sufficient to protect 70% of the vaccinated cattle against clinical disease. But if the match between vaccine strain and circulating strain is poor, one should acquire vaccine with a higher immunogenicity.

When the vaccine is acquired and bought the country should check the immunogenicity of the vaccine by e.g. vaccinating 10 naïve cattle at a state farm. Calves 6 – 8 month old will be in most cases free of maternally derived antibodies and can be used for these tests. Both at the time of vaccination and 3 weeks after vaccination 250 ml blood is collected and after centrifugation serum is stored as reference for later use. Each time new vaccine is acquired the sera of previous batches sera are included in the serological test to see if the immunogenicity is the same.

Similarly one should collect samples in the field from vaccinated animals at the time of vaccination and 3 weeks after vaccination. The immune response in these animals should be similar to the immune response in the cattle vaccinated at the port of entry (the sera of the cattle vaccinated at the point of entry can be included in the test as reference). When the immune response is lower, there is an indication that the cold chain is not well maintained or that the stability of the vaccine is poor.

Another quality control test that should be performed is the determination of the antibody response against non-structural FMDV proteins, after single, two times and three times vaccination. These data are nec-

essary if surveillance programmes use tests for antibodies against non-structural proteins to monitor the risk of FMD in the country (if passive monitoring indicates that clinical disease is absent).

So in post-vaccination monitoring the use of NS tests is not advised except for identifying the possible development of NS antibodies in multiple vaccinated animals. But random sampling of vaccinated animals (preferably twice, at the time of vaccination and 3 weeks later) is essential to monitor the efficacy of the vaccination programme, as stability of FMD vaccines is in general low and failures in the cold-chain can that way be detected earlier.

If vaccine is produced by the country itself it should be controlled as prescribed in the OIE manual to prove that vaccine of a sufficient quality is produced. But challenge tests are expensive and should as quickly as possible be replaced by serological tests when the relationship between antibody response and protection has been established. One should realise that FMD vaccine should induce antibodies against the virus, purification of the antigen to enable the use of NS tests in multiple vaccinated animals should not be pursued as during purification 20 - 40% of the 146S antigen is lost, so the vaccine will become more expensive due to the increase volume of virus that has to be produced and the additional efforts for purification. In our hands a single vaccination with non-purified Frenkel vaccine did not elicit an response in the NS ELISA, so even with non-purified vaccine the NS ELISA can be used in animals that only received a single dose of vaccine. This must be evaluated as stated above.

In this contribution we showed that research on FMD is necessary to be able to control the disease and vaccine quality control is a prerequisite for a successful vaccination campaign.

## Evaluating the effectiveness of foot-and-mouth disease vaccination in the field: learning from human vaccination programmes

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Although over two billion foot and mouth disease (FMD) vaccine doses are thought to be administered each year, little is done to directly measure the extent to which they protect populations against *disease*, relying instead on measures of serum antibody as a correlate of protection and assumed efficacy from the results of vaccine potency tests and matching studies. Given that countries with endemic FMD continue to have outbreaks of FMD despite comprehensive vaccination programmes, it would appear that more appropriate, field-based measures of protection are required.

Whereas potency tests provide evidence of a direct effect of protection to infection with a homologous strain in controlled conditions, the situation in the field is much more complex. Moreover, as po-

tency studies rely on a small number of animals and artificial challenge, results and their wider applicability in the field can be uncertain. Vaccine efficacy towards heterologous strains prevalent in the population can be evaluated through *in vitro* vaccine matching tests. However, none of these experimental assessments account for factors such as differing levels of pathogen exposure, herd immunity effects related to coverage, maternal immunity, number of doses, time elapsed since the last dose, age of animal, and adherence to the cold chain.

As part of the licensing procedure for human vaccines, Phase 1 trials use small numbers of healthy adults to assess the vaccine's safety and any side effects. Some immunogenicity data is also gathered that may indicate the likely efficacy of the vaccine. Phase 2 trials use larger groups of individuals, usually a few hundred, to obtain more information on the immunogenicity and efficacy of the vaccine. Phase 3 (randomised controlled trial) is a further expansion to maybe several thousand individuals with Phase 4 including post-licensure observational studies in particular looking at adverse reactions and ways of optimising use, such as through modification

of vaccine schedules. Phases 2, 3 and 4 trials are performed in the *target population* for the vaccine and therefore give a much more realistic assessment of efficacy or effectiveness. Although equivalent phase 1 and 2 trials are done for veterinary vaccines, large scale studies assessing the protection afforded by FMD vaccines in the field are notable by their absence.

With the support of the European Commission for the Control of FMD (EuFMD), collaborative groups from the Institute of Animal Health, London School of Hygiene and Tropical Medicine and the Royal Veterinary College are applying methods developed for field evaluation of human vaccination programmes to vaccination against FMD.

### Turkey

Vaccine effectiveness studies of a vaccine based on the FMD Asia-1 TUR11 isolate (widely used in Turkey) found that during outbreaks caused by the FMD Asia-1 Sindh08 field virus, although vaccinated animals were not protected against infection, clinical disease was reduced when compared to unvaccinated animals.

Another low potency vaccine based on the FMD Asia-1 Shamir strain did not appear to protect against either disease or infection.

### Kenya

Within Kenya, there are three commonly found serotypes of FMDV



Turkey



Kenya

that appear in continual circulation - O, SAT1 and SAT2. These are seen all year round with an increased incidence during the dry seasons anecdotally due to increased movements of pastoralists at this time. Some areas report up to three outbreaks per year. Although smallholder farmers contribute approximately 80% of the national milk supply, vaccine coverage among this group is poor; although there is occasional government funded reactive vaccination. Despite ring vaccination being a commonly employed strategy for FMD control worldwide, field assessments of the effectiveness of this strategy have not been performed and mostly rely on the results generated from mathematical models. Field based evaluations of reactive vaccination using observational ap-

proaches have been performed for measles and cholera among other human vaccines. Cluster randomised trials provide an excellent method of assessing the effectiveness of such strategies since they capture the full effects of vaccination on the population at large and not just the direct effects of vaccines under field conditions. Not only would this allow the effect of vaccination on

transmission to be assessed but also the cost-benefit for the individual farmer which may inform policy on private-public financing. With a regional approach to FMD control being advocated by several groups, the results of effectiveness studies will have far reaching effects on FMD control in East Africa.

## Foot-and-mouth disease virus in Pakistan and Afghanistan

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### Introduction

In contrast to the situation within Europe and North America, foot-and-mouth disease (FMD) is endemic in many countries especially within Africa and southern Asia. Indeed the problem can be even worse than this statement may suggest since several serotypes of the FMD virus and multiple distinct strains within each serotype can circulate thus making effective disease control very difficult. In Pakistan and Afghanistan, serotypes O, A and Asia-1 of the virus are present and widespread, with FMDV type O usually being the most common (Jamal et al., 2010) but genetic analysis of the viruses in circulation in these countries has been rather limited.

### Characterization of FMDVs in circulation in Pakistan and Afghanistan

In order to determine the extent of virus circulation and to characterize the current FMDVs, as part of an FAO Regional Project (GTFS/INT/907/ITA) over 130 oral swab and epithelial samples were collected from clinically suspect cases in Pakistan and Afghanistan

between July 2008 and August, 2009. These samples were transferred, in an inactivated state, to the National Veterinary Institute, within the Technical University of Denmark (DTU) on Lindholm, Denmark for analysis. The presence of FMDV RNA in these samples was established using real time quantitative RT-PCR assays that targeted sequences within the 5'-untranslated region and the 3D coding region (these assays recognize essentially all strains of FMDV irrespective of serotype). From positive samples, the region of the genome encoding the FMDV capsid protein VP1 was amplified by conventional RT-PCR and the sequences of these amplicons were determined and compared. This analysis can be used to determine the serotype of the virus and also provides information about the genetic relatedness of the different viruses.

From the clinical samples analysed, some 119 VP1 sequences were determined, 33 (28%) were identified using BLAST as serotype O, while 69 (58%) and 18 (15%) were found positive for serotypes A and Asia-1, respectively (note: one sample was positive for both serotypes A and Asia-1). The results from the different serotypes will be considered separately.

### Serotype O

The 33 new serotype O sequences were used in phylogenetic analysis with previously determined sequences of type O FMDVs circulating in Pakistan and Afghanistan since 1997. This analysis

(of some 99 sequences in total) revealed that these viruses belonged to, at least, four lineages within the ME-SA (Middle East South Asia) toptotype (note: toptotypes are defined as geographically clustered viruses that form a single genetic lineage). The four lineages detected were Pak98, Iran2001, PanAsia and a previously unrecognized lineage (Jamal et al., 2011a). In 2008/2009 the PanAsia lineage was predominant and three different PanAsia sublineages have now been detected in both Pakistan and Afghanistan, i.e. PanAsia-I, II and III (the latter was first identified in this region); these sublineages are defined on the basis of >5% difference in VP1 coding sequences.

### Serotype A

Unexpectedly, as indicated above, serotype A was the predominant virus (69 of 119) detected in the clinical samples which were collected in 2008/2009. Using the same procedures as outlined above, the VP1 coding region sequence analyses showed that the sequenced FMDV type A viruses circulating in Pakistan and Afghanistan since 2002 (totaling over 80) belonged to at least four lineages from two distinct genotypes (I and II) within the Asia toptotype, based on the criterion of at least 7.5% nucleotide difference in the VP1 coding region for a separate lineage and 15% for a genotype. Currently, A-Iran05 is the predominant lineage in the region (indeed all but two of the serotype A viruses identified in >60 samples collected in 2008-2009 were in this lineage. However, different sub-lineages within this A-Iran05 lineage have been identified. The dominant sub-lineages were A-Iran05<sup>AFG-07</sup> and A-Iran05<sup>BAR-08</sup> which have both been found previously within the Middle-East. Interestingly, it had been noted by the WRL (Pirbright, U.K.) that vaccines based on the A22/Iraq/64 strain are not expected to protect efficiently against viruses belonging to the A-Iran05 lineage especially the A-Iran05<sup>BAR-08</sup> sub-lineage, this was based on the r1 value determinations.

In order to define the amino acid differences present within the surface exposed capsid proteins of virus strains within the A-Iran05 lineage, the complete nucleotide sequence encoding the capsid proteins (P1) was determined for seven representative strains (selected on the basis of the VP1 sequences). Comparison of the predicted amino acid differences in the exposed capsid proteins (VP1, VP2 and VP3) identified many different substitutions between the A22/Iraq vaccine and the members of the A-Iran05 lineage; significantly, a small number (3) of non-conservative amino acid substitutions (within VP1 and VP3) were identified specifically between the A-Iran05<sup>BAR-08</sup> sub-lineage compared to the A22/Iraq vaccine strain. These amino acid substitutions were mapped onto the known structure of this virus (derived by X-ray crystallography). Two of the changes were highly surface exposed while the third was more hidden but could still affect the outer surface of the virus. Thus these 3 residues are strong candidates for substitutions that can explain the inability of antisera raised against A22/Iraq vaccine to neutralize efficiently the A-Iran05<sup>BAR-08</sup> strains (see Jamal et al., 2011b). This demonstrates the potential utility of complete P1 sequence determination to assist with the understanding and characterization of changes in virus antigenicity.

### Serotype Asia-1

Viruses of the Asia-1 serotype were the least common in Pakistan and Afghanistan in 2008/2009, some 18 new samples were obtained and these were compared to other viruses of this serotype which have circulated in these countries in recent years. Phylogenetic analysis of some 45 strains of FMDV type Asia-1 that have circulated in the pe-

riod 1998-2009 revealed that three different genetic Groups of serotype Asia-1 have circulated in Pakistan during this time. These are Group-II, -VI and, recently, a novel Group (designated as Group-VII). This new Group was not detected in neighbouring Afghanistan at that time (but has been subsequently and it has spread to Iran and Turkey as well) but viruses from Groups I and -II have been in circulation there.

Using near complete genome sequences, from FMDV viruses of serotypes Asia-1 and A from Pakistan, an inter-serotypic recombinant virus has been identified (Jamal et al., 2011c). This has the VP2-VP3-VP1-2A coding sequences derived from a Group-VII Asia-1 virus and the remainder of the genome from a serotype A virus of the A-Iran05<sup>AFG-07</sup> sub-lineage. Such recombinant viruses are derived from co-infection of animals with the two different serotypes and can produce novel viruses with significantly altered properties. Clearly the production of such recombinant viruses relies on the co-circulation of different serotypes of the virus.

### Conclusions

The presence of a complex set of different viruses in circulation within this region clearly creates difficulties for achieving good control of the disease since vaccines have to be matched to the specific viruses which are causing disease. There are also specific features of the farming systems in this region which create good conditions for the spread of the disease. These include the mixing of animals within live animal markets (see photo) and a notable dairy colony of predominantly buffalo at Landhi which may contain 300,000 animals in close proximity. A limited survey of clinically healthy animals entering the Landhi dairy colony involved screening mouth swab samples and sera (Jamal et al., 2012). It was found that 39 of 179 (22%) animals tested on the first day of their entry into the colony (Feb/March, 2009) were positive for FMDV RNA while



Pakistan

130 of them (73%) had anti-NSP antibodies (indicative of previous FMDV infection or use of unpurified vaccines).

It is therefore apparent that control of FMD in such countries still has a long way to go but this work demonstrates that it is possible to identify the nature of the problem which is the basis for movement along the Progressive Control Pathway for FMD which has been adopted by FAO/OIE.

### Acknowledgements

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## Upcoming events:

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29/10/12

### **Open Session of the Eufmd**

Spain

29-31 October 2012

1/11/12

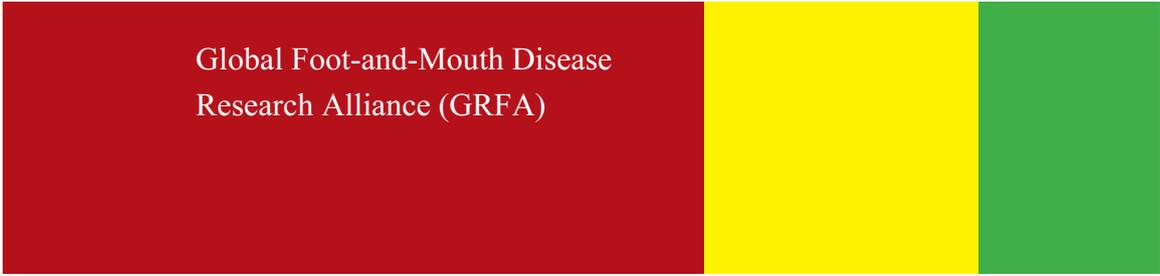
### **Ref. Lab. Meeting**

Spain

1-2 November 2012

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## *Want to Know more:*



Global Foot-and-Mouth Disease  
Research Alliance (GRFA)

### ***The Global Foot-and-Mouth Disease Research Alliance (GFRA):***

*A worldwide association of animal health research organizations to assist the global control and eventual eradication of foot-and-mouth disease*

