

## **1. CRP on the control of foot-and-mouth disease**

### **2. Summary**

Foot-and-mouth disease (FMD) is one of the most important livestock diseases known to man due to its high infection rate (ease of spread) and its effect on the limitation of livestock movement and trade. An outbreak of FMD will have a devastating effect on a country's food security with direct impact on national and international trade. The confirmatory diagnosis of FMD and its effective control through prophylactic, quarantine or slaughter-out procedures are therefore of paramount importance as it has financial and trade implications. Vaccination with inactivated FMD virus is undertaken to control FMD in endemic countries or countries at risk. Vaccines, whilst widely available but which should match (i.e. should be of homologous serotype and strain isolate) with virulent FMD viruses circulating in the region of vaccine use, are of variable quality, not from the homologous outbreak serotype/strain isolate, and are often stored under inadequate temperature conditions and therefore might be not as effective in the field as determined in animal experiments. Due to insufficient knowledge on vaccine strength and antigenic match (antigenic cartography) between vaccine strain and outbreak virus, it is often not possible to pinpoint the weakness of the vaccination strategy and to take action on this weakness.

Vaccine effectiveness can be determined by animal challenge, but this is both costly and difficult. *In-vitro* systems have been developed in different countries since the 1980's, but these are not standardized for international use. Many countries now produce FMD vaccines but often without effective consideration of their effectiveness. This CRP will investigate methods and possibly provide internationally acceptable guidelines for procedures, which test a vaccine's ability to induce the production of protective antibodies in cattle without the need for animal challenge experiments.

In many developing countries, vaccination will continue to be an essential component for the progressive control of FMD. Maximising the effectiveness of current vaccines and supporting research to improve the effectiveness and quality of those and or new vaccines will be critical. Countries using locally produced vaccines need to assure trade partners that they are using quality assured vaccines in order to overcome the restrictive effects of endemic FMD. The provision of internationally accepted guidelines for quality assurance and alternatives to the present need for animal challenge vaccine trials would be a significant step forward. It is likely that control and eventual eradication in endemic areas with a low level resource base (much of Africa, parts of Asia and Latin America) will require the use of quality assured vaccine preparations, correct vaccine formulations (i.e. homologous strain or isolate vaccine to protect against outbreak, new generation vaccines with a broader protection base (i.e. cross protection between different strains and isolates) or alternative formulations of existing vaccines.

This CRP is proposed for five years with three RCM. To apply, please see our website for directions: <http://www-crp.iaea.org/html/faqs.html>.

### **3. Background Situation Analysis**

Foot-and-mouth disease is widely considered an economically important disease of livestock and the single biggest global threat to trade in livestock and livestock products, the latter especially due to its threat to countries where disease is absent. The disease affects most of the major livestock animals of importance (cows, pigs, sheep, goats, and buffalo) in both high intensity/productivity countries and in lower producing, developing countries.

The main threat to areas free of FMD (mostly the developed world) is the immediate consequences on trade in animals and animal products and the subsequent indirect losses through movement restriction of the human population from areas where the disease is present or suspected. The direct losses associated with disease control and re-emergence of

disease into FMD clean areas through destruction of all affected or contact animals or through vaccination are also very high.

In areas endemically infected (most of Africa and Asia and many countries in Latin America) the impact of the disease is not only associated with loss of trading opportunities but also the direct effect on the productivity of the animals through losses associated with milk yield, abortion, death in young animals and loss of traction power. These losses whilst not often dramatic in an endemic setting are insidious and costly. They undermine other attempts at productivity increase and in the subsistence farming setting they are a major factor in maintaining livestock keepers in the poverty trap. Even where vaccination is routinely practised, it is expensive and often ineffective. Vaccination needs to be repeated frequently, vaccine need to protect against currently circulating strains of the virus and should be devoid of contaminating proteins that prevent the use of differentiating vaccinated from naturally infected animals (DIVA) testing. In many developing country settings, this is just not achievable.

FMD is widespread in Asia, India, Africa and certain countries of South America. The world distribution largely reflects the difficulty and ability of countries and regions to control the disease and the relative importance ascribed to the consequences of the disease. A disease with a similar impact, rinderpest, has been successfully eradicated globally and whilst there are a number of differences in these two diseases, the availability of a vaccine that protects against all strains of rinderpest virus and could be given once, was a crucial element in the eradication of rinderpest. Current FMD vaccines are less effective. Setting aside issues of longevity of immunity and inability to protect against all circulating strains of FMD virus, around 50% of FMD vaccinated animals become FMD virus carriers. Whilst the real role of such carrier animals is poorly understood, they represent an unacceptable risk for FMD free areas. A further critical issue in FMD management in endemic areas (whether vaccination is practised or not) is that animal movement must be controlled. This is to ensure that infected animals and the people, who look after them, are kept separate from uninfected stock. This is impractical in many developing countries either because people are poorly educated; because there is a traditional non-acceptance of any authority to control livestock movement or that there is no confirmation that FMD is present through lack of veterinary infrastructure and laboratory diagnostic services.

Despite all the above issues, progressive control in endemic areas will only be achieved using effective vaccines. Any vaccines used in an endemic setting must protect against the FMD virus strain circulating for as long as possible and do so in a way that enables vaccinated animals to be distinguished from naturally infected animals to manage the carrier animal risk. Currently, vaccine assessment is carried out by vaccine and challenge experiments using live animals. This is expensive, difficult and potentially risky in terms of local biosecurity.

At an FAO/IAEA Consultants meeting entitled “Coordinating FMD Research” on 4-7 Dec 2007 in Vienna, an overall conclusion was “Potency testing of vaccines: an international accepted *in vitro* system of vaccine efficacy is needed to avoid challenge protocols”

FMD vaccine production and testing is complicated since:

- a. There are 7 serotypes of FMD viruses (FMDV) that cause the disease in a wide variety of animals. Protection can only be achieved through vaccination using a homologous serotype - this means that effective protection is afforded only when the same serotype of virus is used to formulate the vaccine.
- b. Due to antigenic drift, there exists considerable phenotypic variation between the virus isolate causing the disease in the field and the ‘older’ isolate or reference from the same serotype used to make the vaccine.

- c. The production of FMD vaccines is based on the propagation of live virus in cell-culture, and this leads to disease security problems since large-scale applications are needed (e.g. a BSL type III laboratory is needed for vaccine production).
- d. Propagated FMDV has to be purified and inactivated before formulation to effectively 'kill' the ability of the virus to multiply in animals when administered (since this would cause the disease). At this stage of inactivation, it is vital to maintain the integrity of the virus to allow a good vaccine to be made.
- e. The inactivated virus has to be tested for effective antigenic mass.
- f. The vaccine has to be made through formulating with other substances (adjuvant) to improve the antigen presentation of the virus and improve antibody stimulation.
- g. A good vaccine is one which when injected into animals will induce the production of a high quantity of antibodies that will prevent disease in future. Under the present OIE rules, vaccines have to be efficacy evaluated for conforming protection through challenging vaccinated animals (vaccinated with the test vaccine) with live virus and assessment of the degree of protection. Although there is much opposition by manufacturers and scientists to the rules surrounding the *in vivo* testing of vaccines (e.g. challenging cattle in high containment facilities) and there are highly statistically unfavourable data in its defence, the practice is the only one approved. Due to the importance placed on FMD the rigid rules for vaccine efficacy testing are policed heavily. The development of an international agreement on methods to replace *in vitro* testing is therefore timely.
- h. The industrial scale needed for the production of vaccines warrants a very large investment. A further complication is that countries do not turn to more validated manufacturers for vaccine but make vaccines themselves to try to keep down costs for the sometimes massive numbers of vaccine units needed. Such countries often do not make products where the FMD virus formulated into the vaccine is purified before inactivation and this causes major problems where testing animals as to whether they have been vaccinated or infected comes in during the course of, and after, an outbreak. Although there are tests, which can distinguish on a herd basis whether animals have been infected or vaccinated where purified vaccines have been used, this is impossible with 'unclean' preparations. The CRP D3.20.20 "The use of non-structural protein of foot-and-mouth disease virus (FMDV) to differentiate between vaccinated and infected animals" dealt with such tests.

This CRP proposal will devise quality guidelines to assess (1) and develop protocols and guidelines for application and interpretation of vaccine matching methods (antigenic cartography) to identify the extent of expected cross-protection of FMD viruses, and (2) the degree of contamination of unwanted FMD proteins in vaccine preparations, as well as provide guidelines for assessing the levels of antibodies against the unwanted proteins in vaccinated cattle. It will also evolve methods to distinguish infected and vaccinated animals even where impure vaccines are used. Standards will be agreed for such protocols.

Currently countries free of FMD (mostly in the developed world) protect themselves from the FMD risk by banning the importation of livestock and most livestock products from those areas endemically infected (mostly in the developing world). This polarisation has tended to facilitate research that has highest relevance to developed countries needs given the availability of research funding and where such research is undertaken. A Global FMD Research Alliance has attempted to redress this imbalance as has recent initiatives from the European Union. At a meeting "Coordinating FMD Research" on 4-7 Dec 2007 in Vienna, an overall conclusion was: "The meeting agreed that there is a fragmented research pattern in the world and that there is an intrinsic need to organise coordination meetings between groups, often comprising of the same limited panel of research workers". The meeting fully endorsed the vital need for regular coordination meetings between the relevant parties and recommended that the Joint FAO/IAEA Division could organize a CRP to develop vaccine evaluation systems and the cartographic analysis of FMD isolates.

FAO in 2007 launched a global program aimed at the progressive control and eventual eradication of FMD. It is clear that vaccination will have a crucial part of play in the control of FMD in endemic settings and in any opportunity for eradication. Linking the outcomes of this CRP in terms of current vaccine quality control protocols, with research on new generation vaccines will be crucial to these FAO aims and the RCM component of FAO/IAEA CRP is an ideal vehicle for achieving this.

#### **4. Nuclear Component**

**This CRP will involve the following nuclear components:**

- Rapid, sensitive and specific detection of disease agent nucleic acids using molecular technologies (e.g. reverse transcription polymerase chain reaction (RT-PCR) and PCR sequencing), applying the use of isotopes ( $P^{32/33}$ ,  $S^{35}$  and  $S^{35}Met$ ) to label PCR amplicons during development and comparative phases of research, and for the evaluation or characterization of targeted genes.
- Use of nuclear associated and nuclear related techniques, where the technologies involve nuclear components, such as ELISA, as well as radio isotopes in molecular studies on FMD virus strains for use in and included in vaccine formulations and in exploiting the PCR for gene amplification in genetic sequencing.

#### **5. Overall Objective**

The CRP is linked to project 2.1.2.3: Molecular technologies for improving productivity in smallholder livestock systems. To develop guidelines and protocols for the more effective quality control of FMD vaccines and their application in endemic countries as part of the FAO program for the global progressive control and eventual eradication of FMD in domesticated animal reservoirs.

#### **6. Specific Research Objectives**

- a) Establish methods and develop internationally agreed protocols for measuring the potency of FMD vaccines using *in vitro* methods.
- b) Establish guidelines for optimum population vaccination intervals based on *in vitro* measurements of potency and duration of the antibody response to structural proteins, after vaccination of cattle and small ruminants with commercially available FMD vaccines, including evaluation of reduced dose options such as intradermal administration of FMD vaccine;
- c) Establish protocols and guidelines for application and interpretation of vaccine matching methods (antigenic cartography) to identify the extent of expected cross-protection of type A or SAT viruses
- d) Provide further global co-ordination of current research into FMD vaccines for use in endemic settings

#### **7. Expected Research Outputs**

- a) Detailed evaluation of currently available methods for the measurement of FMD vaccine potency using in-vitro methods and comparison of these with FMD in-vivo challenge protocols.
- b) The selection of the most efficacious in-vitro method and the development of this into an internationally agreed protocol for use in a developing country setting for the quality control of FMD vaccines, and evidence based guidelines for vaccination interval based on kinetics of antibody responses to vaccination in endemic area settings,
- c) Standardised protocols and biological reagents that will enable the widespread application in DC laboratories of antigenic cartography methods for assessment of suitability of locally and international produced vaccines against epidemic FMDV,
- d) An evaluation of the use of DIVA tests for assessing the presence of FMDV non-structural proteins (NSP) in locally available vaccines,

- e) Annual assessment report on FMD research at a global level focused on vaccine improvement and development highlighting areas of improved co-ordination and potential impact for FMD endemically infected,
- f) Annual report linking the specific research outputs of this CRP with the overall global research effort as outlined in D) above.

### **8. Expected Research Outcomes**

- a) Internationally recognised methods and SOPs to allow the quality control of vaccines in terms of potency, expected level of cross-protection, and induction of NSP.
- b) Internationally recognised methods (OIE and FAO endorsed), SOPs and guidelines for optimising vaccination programmes based on the kinetics of responses to FMD vaccines, and the extent of cross-protection. This will lead to improved vaccine efficacy and use of vaccines to generate population immunity, and ultimately the more effective control of FMD in endemic settings. The possibility to substantially reduce the vaccine dose by alternative vaccination protocols may allow countries to make major savings or to extend the population coverage for the same investment.
- b) A more globally coordinated approach to research on FMD vaccines and their use

### **9. Action Plan (Activities)**

A1. Award research contracts aimed at achieving the research outcomes detailed in the proposal; Award agreement holder contracts both from commercial sources as well as from Research Institutions.

B1. Develop protocols to evaluate strain antigenic cartographic limitations, and develop and evaluate FMD vaccines and their quality.

C1. International meeting(s) on FMD research activities (donors included).

#### 9.1. Organisation of the CRP network

CRP Research contract holders from IAEA and FAO Member State national laboratories with a track record of FMD control achievements and where FMD is a national priority.

Agreement holders from Commercial producers involved in vaccine quality control and vaccine control organizations (i.e. AU/PANVAC).

#### 9.2. First RCM in 2011

Discuss workplan (s) in detail and involve possible donors and commercial companies from the very beginning (note the consultant's meeting recommendations already). Discuss current situation with regard in vitro testing of animals and assessment of NSP contamination linked to NSP antibody testing.

Allocate research tasks and management scheme for each set tasks to allow reporting.

Discuss FMD research and formulate coordination plans.

#### 9.3. Second RCM 2013

#### 9.4. Third RCM 2014