EPIZONE

www.epizone-eu.net

CENTRAL VETERINARY INSTITUTE
WAGENINGEN UR

Central Veterinary Institute of
Wageningen UR
P.O. Box 65
8200 AB Lelystad, Netherlands
Houtribweg 39
8221 RA Lelystad, Netherlands
+31.320.238800
epizone.cvi@wur.nl

Minutes of EPIZONE workshop "FMDV Minimum Standards Implementation"

EPIZONE: Network of Excellence for Epizootic Disease Diagnosis and Control.This network is supported by funding under the Sixth Research Framework Programme of the European Union'.

Partners of EPIZONE: CVI Wageningen UR (NL), Friedrich-Loeffler-Institute (D), Institute for Animal Health (GB), Veterinary Laboratories Agency (GB), Agence Française de Securité Sanitaire des Aliments (F), Technical University of Denmark, National Veterinary Institute (VET-DK), Statens Veterinarmedicinska Anstalt (S), Centre de coopération Internationale en Recherche Aronomique pour le Développement (F), Center of Animal Health, National Institute for Agriculture and Food Research and Technology (E), Instituto Zooprofilattico Sperimentale delle Venezie (I), Lanzhou Veterinary Research Institute (CN), National Veterinary Research Institute (PL), FMD Institute Ankara (TR), Veterinary and Agrochemical Research Centre, VAR-CODA-CERVA (B), Hannover Veterinary School (D), Instituto Zooprofilattico Sperimentale della Lombardia e dell' Emillia Romagna Brescia (I), Harbin Veterinary Research Institute (CN), Food and Agriculture Organization (I), DiVa Digital Value (NL)

Central Veterinary Institute, part of Wageningen UR

DATUM
20 januari 2012

POSTADRES
Postbus 65
8200 AB Lelystad

BEZOEKADRES
Gebouw 211
Houtribweg 39
8221 RA Lelystad

INTERNET
www.cvi.wur.nl

KvK NUMMER
09098104

CONTACTPERSOON
D. Kuperus

TELEFOON
+31 (0)320-238840

E-MAIL
douwe.kuperus@wur.nl

Program workshop
"FMDV Minimum Standards implementation",

Introduction (all)

Discussions:

1. Inactivation / testing of samples to be shipped (introduced by Summermatter)
2. Integrity test of HEPA-filters (Müller-Doblies)
3. Role of competent authority (Haas)
4. Quarantine-regulations (Kuperus)
5. Project "AniBioThreat" (Peeters)

Any Other Business

Minutes of EPIZONE workshop
"FMDV Minimum Standards Implementation"

January 27th 2012, Central Veterinary Institute, Lelystad, the Netherlands

Attendants:

Ben Peeters	CVI The Netherlands (*chair*)
Kirsten Tjørnehøj	DTU Denmark
Uwe Müller-Doblies	IAH United Kingdom
Kathrin Summermatter	IVI Switzerland
Bernd Haas	FLI Germany
Koen Quanten	CODA Belgium
Annebel de Vleeschauwer	CODA Belgium
Cesare Berneri	ISZLER Italy
Sébastian Allix	ANSES France
Douwe Kuperus	CVI The Netherlands
Heleen Klos	CVI The Netherlands
Randi Buijs / Heleen Klos	CVI The Netherlands (*minutes*)

Introduction

On April 30th 2009, the document "MINIMUM STANDARDS FOR LABORATORIES WORKING WITH FMDV *IN VITRO / IN VIVO*", hereinafter the 'Minimum Standards', was adopted by the 38th General Session of the European Commission for the Control of Foot-and-mouth Disease (EuFMD). The Minimum Standards describe a set of procedures and precautions to be taken by laboratories and institutes that handle live Foot-and-mouth disease virus (FMDV).

At the initiative of the Central Veterinary Institute, a workshop was organized on January 27th, 2012, in Lelystad, The Netherlands. The purpose of this meeting was to bring together the Biosafety/Biorisk officers of the European institutes that handle live FMDV, in order to discuss the implementation of the Minimum Standards.

The workshop started with a personal introduction and a brief overview of the laboratory and research facilities by each of the participants. Next, the agenda was followed, with the addition of two additional items which were brought in by Cesare Berneri (digestion of animal carcasses) and Koen Quanten (requirements for airtightness of new containment buildings).

1 Inactivation / testing of samples to be shipped (Summermatter; annex 1)

Paragraph IX, *Equipment and Materials*, of the Minimum Standards describes the "*Removal of biological material from the restricted area*". Articles 67 and 68 state that the necessary precautions must be taken to ensure that the material does not contain FMDV. However, at this moment there are few standardized methods available and criteria for the validation of these methods do not exist. In her introduction, Kathrin Summermatter (IVI) outlined the large variation in the type of biological material (nucleic acids, blood, cell cultures, serum, tissues, etc.). Whereas some of these materials can be treated by heat or chemicals to inactivate FMDV, others cannot and have to be tested for the absence of FMDV by an in vitro cell-based innocuity test or PCR. Although each of the participating institutes has procedures in place to perform either inactivation or innocuity testing, these procedures have not been harmonized between the different institutes. Validation is based on own experience and criteria, but no proficiency testing (ring trial) is performed.

Different materials may originate from different areas or compartments within a high containment unit, each with a different risk of being cross-contaminated by FMDV. Material may come from a laboratory where FMDV is handled, from an FMDV free laboratory, from FMDV infected animals, or from other animals, infected or not with other pathogens. Should these samples be treated similarly, or is it possible to differentiate between the required stringency and sensitivity of the different inactivation and test procedures? Other questions raised include: i) is the material being sent to an institute that maintains the same biosafety level, ii) what about the duties of the receiver, iii) will the receiver comply with the requirements of the sender? Another important point to consider was the intended use of the material (e.g. laboratory, animal experiment, etc.). The participants agreed that all of these questions are relevant but that it is difficult to come up with general procedures. Specific situations may ask for specific procedures which are not covered by existing procedures.

As a start to try and harmonize at least some of the procedures/protocols used by the different FMD-labs, it was agreed to share these procedures by putting them on a shared website of the International Veterinary Biosafety Workgroup (IVBW) where everyone can view them. One of the problems is that not all protocols are available in English and that some protocols apply to special local situations. Therefore, it was agreed to submit a short outline of each protocol in English which will probably suffice to make a comparison. Furthermore, the participants expressed the need for a risk matrix based on the potential or expected contamination level of the compartment from which the samples originate.

The participants agreed that detection of FMDV genome sequences by RT-PCR is probably as sensitive, and certainly more convenient, than in vitro innocuity tests. However, the minimum percentage of the sample to be tested and the limit of detection of the different PCR protocols used by each lab should be agreed upon. The performance (limit of detection) of different tests should be evaluated in ring trials.

Finally, there is a need to agree on the contents, including liability, of the acceptance forms (materials transfer forms) that are sent to the receiving parties.

2 Integrity test of HEPA-filters (Müller-Doblies)

Paragraph VII of the Minimum Standards describes the "*Air Handling – Live Virus Facilities*" (articles 43-52). Uwe Müller-Doblies (IAH) indicated that there are no well-defined criteria for the testing of HEPA filters and e.g. no limiting values described for the permeability of a HEPA filter. Part 48 of this paragraph only describes: "*When HEPA filters are installed or replaced, an in-situ efficiency test must be carried out by trained personnel with validated equipment.*"

One of the first points of discussion was the question how reliable HEPA filters are for their intended purpose, i.e. the retention of infectious FMDV. Few - if any - systematic studies have been conducted to assess the behaviour and viability of FMDV particles in air, let alone in HEPA filters. The efficiency of HEPA filters is very high but not 100%. This is probably dependent on the test system, but FMDV itself has not been used for such tests.

Many questions remain. Can we guarantee that all FMDV will be retained? When testing HEPA filters, which criteria should be used for the '*in-situ efficiency test*' mentioned in the Minimum Standards? Different institutes use different filter systems (HEPA 13 and 14) from different suppliers. Which one should be the minimum standard? What equipment, and which procedures and criteria (cut-off values) should be used to perform the filter test? Is an overall filter integrity test sufficient or should we use a scanning device (T-bar scanner)? What particle size should the smoke generator produce and should we use a photometer or particle counter to measure the integrity and efficiency of the filters? Different countries also use different precaution methods (see also *Quarantine regulations* below). Different risk zones are installed by different institutes.

In conclusion: specific standardized criteria for the minimum efficiency to which HEPA filters should comply must be defined. Also, the different methods that are currently being used to perform the in-situ efficiency test must be validated in order to guarantee the safety of the environment.

3 Role of competent authorities (Haas; annex 2)

Article 65 of COUNCIL DIRECTIVE 2003/85/EC of 29 September 2003 on *Community measures for the control of foot-and-mouth disease* states that '*Member States shall ensure that laboratories and establishments in which live foot-and-mouth disease virus, its genome, antigens or vaccines produced from such antigens are handled for research, diagnosis or manufacture, are strictly controlled by the competent authorities*'. Bernd Haas (FLI) shared his experiences as a member of the Food and Veterinary Office (FVO) inspection team involved in audits of FMD-labs throughout Europe. In his introduction he indicated that, in most countries, article 65 has not yet been fully transposed into the national legislation. Furthermore, he expressed his concern on the role of the competent authorities in relation to their responsibility towards the FMD-laboratories. Because the inspectors of the competent authorities are not subjected to the pragmatic laboratory work, there is no specific training or knowledge with respect to FMDV. Therefore, he proposed that technical part of the inspection of FMD-labs should be executed by delegates of an expert group at the European level. In order to install such an expert group, the EuFMD Research Group should be consulted.

Haas also proposed to split up the Minimum Standards according to risk level:

1. Minimum Standards for "real" FMD-labs (i.e. research labs and diagnostic labs working with live FMDV in 'peace time')
2. Minimum Standards for "auxiliary" FMD-labs (i.e. national diagnostic reference laboratories working with suspect samples from their own country, without using live FMDV as a reagent).

Currently, an annex to the Minimum Standards covers auxiliary labs that could support national labs in times of outbreak. Müller-Doblies proposed to write a project, with the aim to get funding to define the Minimum Standards for auxiliary laboratories (such as the ex SU countries).

In conclusion: the FVO audits have shown that the inspectors of the competent authorities lack the necessary expertise and specific knowledge to execute the technical part of the FMDV audits. Therefore, the instalment of an expert group at the European level that executes the technical part of the inspections should be considered.

It is important that small countries are able to perform FMD diagnosis. Since the requirements of the Minimum Standards do not always apply for diagnostic laboratories that do not use live FMDV as a reagent, it should be considered to develop separate minimum standards for such auxiliary labs.

4 Quarantine requirements (Kuperus; annex 3)

Douwe Kuperus (CVI) introduced another difficult item: Quarantine (Paragraph IV, article 27 and 28 of the Minimum Standards). According to paragraph IV, for personnel the following restrictions apply: '*... each facility must define and apply quarantine periods for persons authorised to work in each category of controlled zone/restricted area*' and '*... abide by minimum standards of quarantine i.e. no contact with animals susceptible to Foot-and-Mouth Disease for at least three days*'. Both article 27 and 28 leave quite some room for interpretation. For instance, article 27 states that the range of the quarantine period may depend on the level of exposure to virus. In article 28, what is the definition of '*contact*' with FMD susceptible animals? Are people not allowed to touch them? Do they have to stay away 3, 5, or 10 meters, or even more? Are people not allowed to visit a farm at all, or is it allowed to go into the farmhouse if the cattle is kept in a separate stable on the same premises?

In the Netherlands, CVI Lelystad is interpreting the quarantine requirements by a legislative approach without differentiation, i.e. all visitors of the restricted area must sign a declaration in which they agree not to visit a *location* with FMD susceptible animals within 72 hours. Furthermore, it is not allowed to keep FMDV-susceptible animals within a radius of 3 kilometres around the institute. Other institutes interpret the quarantine-requirements differently and this often leads to questions by the people concerned.

In Germany (at the FLI), there are basically two rules:

Persons must not enter stables or enclosures where susceptible animals are kept for three days after leaving the FMD containment area ('BSL3^{+}').

In addition, no premises where susceptible animals are kept must be entered for 7 days after leaving the FMD containment area ('BSL3^{+}'). However, the second rule does not apply to the premise of the FLI Riems and may be modified according to risk-assessment on a case-by-case basis in respect to other premises. Otherwise, a complex premise with different containment zones like the FLI Riems

could not be managed and many scientists couldn't give talks at e.g. universities where susceptible animals are kept somewhere at the premise.
Denmark has different rules for workers in the animal room or laboratory workers. For animal rooms with FMDV-infected animals the quarantine period is 5 days, for laboratory workers not handling live FMDV the quarantine period is 3 days, while guests get 7 days of quarantine. It is forbidden to go stables or enclosures where susceptible animals are kept, including e.g. zoos and fenced deer, within the quarantine period. However, farm houses and gardens without cloven-hoofed animals can be entered.
In England several different quarantine periods apply, depending on the work performed and the involvement of live FMDV. After the FMDV outbreak in 2007, it was decided that there will be an animal-free area around the laboratories. If there is an encounter with animals at risk, people must try to stay away from the animals as far as possible.
In Switzerland, the FMD-lab (IVI) is surrounded by meadows where cattle and sheep may freely graze. The only rule they advise to lab workers is "*to walk on the other side of the street*" and to avoid contact with susceptible animals. Access to some parts of a farm house is allowed if people have not worked with FMDV and direct contact with FMDV susceptible animals is avoided. However, during FMDV experiments stricter rules apply!
In Italy the quarantine rules state that cattle have to stay away from the facility, and personnel away from cattle, for at least 25 meters. At ISZLER they apply three days quarantine for personnel working in laboratories (diagnostic) and five days for stables and small productions.
The above examples indicate that there is no uniformity with respect to the quarantine rules between the different European FMD-labs. Therefore, it would be worthwhile to include more specific definitions with respect to the quarantine requirements in the Minimum Standards. Perhaps these requirements could follow the above suggested risk matrix based on the expected contamination level of the working environment.

5 Project "AniBioThreat"

Due to time limitations, point 5 "Project AniBioThreat" by Ben Peeters was suspended.

Briefly discussed: ***Criteria concerning airtightness of the new building.***

Currently in Brussels a new containment-building is being constructed. Quanten asked for information with respect to the airtightness-requirements of such a building. Several participant explained their experiences and explained which criteria they use and how to test this. However, specific requirements are not included in the Minimum Standards.

The issue of digestion of animal carcasses was not discussed due to time limitations.

Closing

Peeters and Kuperus thank their colleagues for participating in this workshop. All attendants agreed that this was a very useful meeting that deserves a follow-up, if possible on a regular basis. Such a meeting should include all European FMD-labs working with live FMDV in association with susceptible animals. Unfortunately, our colleagues from Spain (INIA, Valdeolmos) could not make it this time. Hopefully they will be able to attend the next meeting. In order to arrange follow-up meetings, the participants will try to apply for funds in order to cover the cost of travel and subsistence.

A copy of this document is available on the Epizone-website (*www.epizone-eu.net*)

Federal Department of Economic Affairs FDEA
Federal veterinary office FVO

Inactivation of samples Sample transfer

Summermatter Kathrin
Leylistad, 27 January 2012

Introduction

- Overview
- Type of material
- Containment – procedures
- Information about samples
- Inactivation – inocuity testing of samples
- Removal from containment
- Shipment
- Duties of receiver
- Questions

Overview

- EU FMDV standard: Removal of biol. Material from restricted area, points 67. / 68
- No standardized methods available
- No criteria for validation of the methods
- Not only FMDV but also other risk group 3 and 4 organisms used
- Limitations: sensitivity of test – quantity of material to be tested
- Different type of materials which have to be brought out of containment (high risk – low risk)
- Different layouts of FMDV containment laboratories and animal units
- Responsibilities of receiver and sender
- Further use of sample (laboratory – animal)

Type of material

Laboratory - animal unit :

- Nucleic acids / replicons (DNA, RNA)
- Proteins
- VLP / virosomes
- Cell cultures
- Virus cultures (FMDV, others)
- Blood
- Serum
- Plasma
- Organs
- Tissues
- Nanoparticles

Containment – procedures

- Containment unit with individual laboratories and animal stables:
  - Material from a laboratory where FMDV work is done
  - Material from an FMDV free laboratory, but inside the FMDV containment
  - Material from FMDV infected animals
  - Material from other animals, infected or not with other pathogens
- Containment unit with interconnected spaces: 1 zone
- Decontamination procedures
- Personal protective equipment
- Degree of contamination of material (e.g. pipettes, consumables etc.)

Information about samples

- Origin
- Function – sequence
- Description
- Method of inactivation – buffer in which the sample should be removed from the containment
- Responsible person
- Receiver
- For nanoparticles: coating material

Inactivation – inocuity testing of samples

- No inactivation for samples which will be sent to the same containment level
- Inactivation for laboratories with lower containment levels
- Different methods:
  - BPL
  - BEI (Biethyleneimine)
  - Formaldehyde fixation
  - Heat
  - Other (?)
- Inocuity testing of samples:
  - RT-PCR
  - Cell culture (how many passages?)
  - What % of the sample?

Removal from containment

How?
- Dunk tank
- Fumigation airlock (H2O2, formaldehyde, else)

In which tubes?
- Nunc tubes
- Eppendorf (?)
- Tupe with seal

Tracability – log book:
- Which information?
- Who is in charge?

Duties of the receiver

- Provide appropriate containment level
- Possess the license to work with the material
- Liability – sign statement
- Treat material as indicated by sender (e.g. no use of material in animals, appropriate treatment of contaminated material etc.)

Examples

Spleen from mice for hybridoma
- Mice cage in the animal unit but in separate room, no change of cloth
- DNA and RNA extraction
- RT-qPCR (FMDV, CSFV, AHSV, ASFV)

Organs and tissue:
- 4% formaldehyde solution
- Samples max. 1-2cm3
- Fixation mind. 24h

Plasma (low risk samples):
- BEI inactivation using positive controls (use replicate tube spiked with 10^6 $TCID_{50}$); one cell passage

Nucelic acid: Phenol – heat

Questions

- Do we have to treat all the samples in the same way? (samples from FMDV infected animals (high risk samples), samples from animals infected with other viruses (medium risk), virus free samples (low risk)
- Are there standardised methods (e.g. BEI, heat inactivation) for the different materials?
- How likely is it that a sample which is handled inside an FMDV containment is contaminated with FMDV?
- Limitation of tests: Sensitivity vs. amount of samples to be tested?
- How many cell passages for which material?
- What information is required from the receiver?
- What is the evidence / scientific basis?
- Containment level? BSL4vet – BSL3Ag – BSL3?

TO DISCUSS:

Can we share protocols for different types of material?

Liability forms?

Material transfer agreement content?

Bernd Haas, NRL for FMD, FLI Riems
Institut für Virusdiagnostik – Institute of Diagnostic Virology

FVO-INSPECTIONS

In accordance with Article 66 of Directive 2003/85 EC, in 2009-2011 the FVO has inspected

3 FMD vaccine manufacturers listed in Part B of Annex XI to the Directive (UK, NL and DE)

9 FMD laboratories listed in Part A of Annex XI to the Directive (NL, UK, DE, BG, BE, DK, PL, ES and EL).

2012: 7 remaining laboratories (CZ, AT, RO, HU, FR, IT and LT).

LEGISLATION

Legal requirements
Article 93 of Council Directive 2003/85/EC requires Member States to bring into force the laws, regulations and administrative provisions necessary to comply with the Directive.

Common Problem
Article 65 of Council Directive 2003/85/EC has not been fully transposed into the national legislation.

COMPETENT AUTHORITIES

Legal requirements I

Article 65 of Council Directive 2003/85/EC requires the Member States (MS) to ensure that:

(a) laboratories and establishments, in which live foot-and-mouth disease virus, its genome, antigens or vaccines produced from such antigens are handled for research, diagnosis or manufacture, are strictly controlled by the competent authorities;

(b) the handling of live foot-and-mouth disease virus for research and diagnosis is carried out only in approved laboratories listed in Part A of Annex XI;

COMPETENT AUTHORITIES

Legal requirements II

Article 65 of Council Directive 2003/85/EC requires the Member States (MS) to ensure that:

(c) the handling of live foot-and-mouth disease, virus for the manufacturing of either inactivated antigens for the production of vaccines or vaccines and research, is carried out only in the approved establishment and laboratories listed in Part B of the Annex XI.

(d) the laboratories and establishments referred to in points (b) and (c) are operated at least according to the biosecurity standards set out in Annex XII.

What is checked in particular and what common problems were found?

1. Designation of the competent authority
   Often unclear, ministry or local or regional authority without specific knowledge on bio-risk management
2. Approval of the laboratory to handle live FMD virus
   Often no specific approval for FMD work

What is checked in particular and what common problems were found?

3. Organization of official controls
   Often no structured approach
   In 10 out of the 11 facilities inspected these controls were not carried out or did not sufficiently cover FMD bio-risk management
   If there was good bio-risk management, it was due to an internal structure!
4. Enforcement powers
   Usually in place
5. Notification procedures in case of emergencies
   Often on an ad hoc basis

What is checked in particular and what common problems were found?

6. Qualification of the CA inspectors
   Entirely inadequate to inspect the complex bio- risk management systems,
   in particular the technical installations of the air ventilation systems and the effluent treatment plants

   Inspectors usually come from local or regional authority without specific knowledge on bio-risk management

General Problem in some facilities

Staff running e.g. waste water treament plants and autoclaves

vs

Scientists with bio-risk responsibilities

(reality vs paper)

What should be done about it?

Delegate technical part of inspections to an expert group at European level

Consult:
EuFMD Research Group

FMD laboratory Bio-Risk Officers (BRO)

Further suggestions:

Split up "Minimum Standards" according to risk:

"Real" FMD labs doing research and diagnostic work on foreign samples which contain or may contain live FMDV "in peace times"

"Auxiliary" labs investigating only suspect samples from own country without using live FMDV as a reagent

This would then cover also some of the "national labs" Currently, an annex to the Minimum standards covers auxiliary labs that could support national labs in times of outbreak

Quarantine-questions

Douwe Kuperus

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

MINIMUM CONTAINMENT STANDARDS FOR FMD LABORATORIES

- Quarantine: each facility must define and apply quarantine periods for persons authorised to work in each category of controlled zone/restricted area, to reduce the risk that personnel will cause a release of FMD virus as a result of virus carriage on their body. A range of quarantine periods depending on the level of exposure to virus. Depending on the risk assessment application of quarantine rules may be applied to other areas of a facility as well.
- Persons, including visitors, authorised to enter the FMDV restricted area must agree not to keep any animals which are susceptible to FMD, nor reside on premises where such animals are kept and to abide by minimum standards of quarantine, i.e. no contact with animals susceptible to foot-and-mouth disease for at least **three** days.

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

From the visitor declaration CVI:

- Within 72 hours after leaving the khaki or negative pressured zone you are not allowed to visit any poultry plants or locations with animals susceptible to FMD. Susceptible to FMD are i.e. cattle, pigs, sheep, goats, deer, elephant and giraffes. These animals can for instance be present in a (children's) farm, zoo, livestock market or animal facilities.

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

Does 'contact' mean: a visit to the farmhouse, the farmers house, the fields, etc.?