

Detection of FMD Infection in Vaccinated Animals

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The purpose of this paper is to briefly review the state of development of tests for the detection of FMD infection in vaccinated animals and the guidelines that are available for their validation and ultimate use. A brief update will be given on studies to develop and evaluate such tests at IAH-Pirbright, in connection to a recent vaccine-challenge experiment.

The most direct method to detect FMD infection in vaccinated animals is to look for the FMD virus. However, the acute phase of virus replication may be transient and in the absence of clinical signs, the chance of detecting this is small. The proportion of vaccinated ruminants that go on to become persistently infected is variable as is the duration of virus carriage. Persistently infected animals (sometimes referred to as “carriers”) can be identified by virus isolation or RT-PCR tests on oropharyngeal fluid (probang) samples, but the method has a low diagnostic sensitivity (Haas and Sorensen, 2002). Studies in South America found that virus could only be detected intermittently from serial oropharyngeal fluid samples (47% positive) collected from 78 persistently infected animals that scored consistently seropositive in tests for antibody to non-structural proteins (NSPs) (Bergmann, personal communication). Another serious limitation of oropharyngeal fluid testing and subsequent virological tests is the difficulty of carrying out both procedures rapidly on a large scale. Tests for virus on oropharyngeal fluids can be carried out to try to confirm the status of animals identified as potentially persistently infected by serological examinations. However, only a positive result adds much weight to the diagnosis because of the high likelihood of achieving a false negative result. Furthermore, if serological tests are required to be used on a herd-basis, then herds testing positive should have all of their animals tested by the confirmatory method, and not just the individual seropositive animals.

There has been considerable progress in the establishment of commercially available serological tests for antibodies to NSPs (sometimes referred to as FMD marker tests). The OIE Diagnostic Manual recommends the use of the South American 3ABC “PANAFTOSA” ELISA and confirmatory western blot technique (enzyme-linked immuno-electrotransfer blot or EITB) (OIE, 2000; Bergmann et al., 2000). This system has been used on by far the largest number of samples, albeit mostly from cattle and is the approach that the OIE’s ad hoc working group have recently recommended to be considered as the reference (or index) method to which others should be compared (OIE 2002). These tests are considered to have been validated in cattle and to have a high diagnostic sensitivity and specificity (OIE, 2002). The validation data presented to the OIE ad hoc working group included a very large number of results from serial samples collected from persistently infected cattle. However, these included field sera that had

been pre-selected on the basis of reactivity in the test under evaluation or came from experimental animals that had been infected without prior vaccination. There are questions over the availability of the test to laboratories in Europe and over the ease of interpretation of the western blot technique. Preliminary studies carried out by European laboratories have not confirmed the high specificity of the 3ABC “PANAFTOSA” ELISA test reported in South America (Dekker, Haas and De Clercq, personal communication).

There are two commercially available tests for antibodies to FMD NSPs (Fig 1). One is the FMD-CHEKIT-ELISA from Bommeli (Schalch et al, 2002) and the other is the UBI FMDV NSP ELISA (Liu et al., 2002). Both of these are indirect ELISAs requiring different conjugates (and therefore different kits) for the examination of sera from ruminants and pigs. Both tests are easy and quick to perform, although both involve a pre-dilution step in the preparation of the test serum, which significantly adds to the work involved when testing very large sample numbers. Whereas the Bommeli test employs a recombinant 3ABC antigen directly coated to the ELISA plate, the UBI test employs a 3B peptide. UBI can also supply two additional peptide-based tests which can be used to confirm the result obtained with the 3B indirect ELISA. One is a blocking test, in which sera reacting in the 3B test are incubated with free 3B peptide. The other is an indirect ELISA with a 3A peptide coated the wells of the ELISA plates. Another test that is expected to become commercially available is the Ceditest FMDV-NS from Cedi Diagnostics. This is a blocking ELISA based on the method of Sorensen (Fig 1; Chung et al., 2002). Data pooled from different European laboratories on testing a rather small number of 15-75 persistently infected cattle that had been experimentally inoculated with FMDV after vaccination suggested that the sensitivity of detecting such animals was approximately 70%, 80% and 90%, using the UBI, Bommeli and Sorensen methods respectively. It should be emphasised that not all sera were tested with all of the assays and the data on the Sorensen test relates to the original method and not the newly produced test kits from Cedi Diagnostics.

Other NSP tests under development include additional 3ABC ELISAs, other peptide-based ELISAs using 3B (Grunmach et al., 2000) or 2B (Inoue, personal communication) and 3D-based ELISAs. Alternative methods to detect FMD infection, post-vaccination, include the detection of specific IgA in oral secretions and the use of multiple antigens presented in a different format to that used in the western blot, for example some form of micro-array. To date, rather variable results have been obtained using different methods of IgA analysis, but only small numbers of samples have been examined. It would also be worthwhile to evaluate cytokine and cell mediated responses as a basis for specifically detecting the immune response due to infection. Pen-side versions of tests for the detection of infection-specific responses would also be very useful and various methods are under development.

Despite considerable interest in better FMD marker tests, the numbers of recent publications on the subject are few. Those involved in the development and evaluation of tests for the detection of FMD infection in vaccinated animals are frequently asked why validation of new tests is rather slow. Three contributory factors are (1) the requirement

for a very high degree of certainty in the information on test performance, (2) the complexity of the different scenarios for test use that have to be evaluated and (3) the lack of sera from known vaccinated and infected animals.

In May 2002, the OIE approved a new set of guidelines that expands on the chapter in the OIE Diagnostic Manual and the paper of Jacobson (1998). This defines a validated assay as a method that “consistently provides test results that identify animals as positive or negative for a particular analyte or reaction which, by inference, accurately predicts the infection status of the animal with a predetermined degree of statistical certainty”. The stages of assay development and validation are described in some detail and examination of these reveals a number of critical issues for NSP validation. Firstly, there is a requirement to know the purpose of testing and the expected prevalence of the infection to be detected. This is needed in order to calculate the sensitivity and specificity requirements for any tests. However, for FMD NSP tests, there is a lack of information on the expected within-herd prevalence of persistently infected animals in post-vaccination populations. Consequently, requirements for sensitivity and specificity have not been accurately determined. Other problematic issues are the lack of reference standard sera, the lack of a readily available index method and the difficulty of fulfilling the requirement to test sera obtained from at least 300 individual persistently infected animals. The International Atomic Energy Association (IAEA) has also been involved in establishing requirements for the validation of NSP tests (Crowther, 2001) and held a workshop to review progress on this subject in March 2002.

Some guidance on the usage of NSP tests is available in the OIE Terrestrial Animal Health Code (2003) (http://www.oie.int/eng/normes/MCode/A_00157.htm) and in the new EU FMD Directive (Anonymous, 2003). The OIE code establishes the criteria for returning to a FMD-free status, of which two of the available options require NSP serosurveillance, i.e. (1) Recovery of FMD-free status without vaccination, after at least 6 months, by stamping out, emergency vaccination and post-vaccinal serosurveillance. (2) Recovery of FMD-free status with vaccination, after at least 18 months, by vaccination plus post-vaccinal NSP serosurveillance. Further guidance is to be found in the OIE Preliminary Guidelines for the establishment or the regaining of recognition for a FMD-free country or region (with or without vaccination). However, it is not always clear which guidelines are applicable to which control and surveillance scenarios. The EU Directive sets out three phases to vaccination: (1) from the start of vaccination until 30 days after the last vaccination. (2) From 30 days after vaccination until the completion of the serosurveillance. (3) From the completion of serosurveillance until the restoration of the FMD-free status. However, the within herd sampling criteria appear ambiguous with alternative options to sample at a level to detect 5% of infection with 95% confidence or to sample all vaccinated animals and their unvaccinated offspring in all vaccinated herds (in all vaccinated herds).

Provisional results are available from a recently conducted comparison, carried out at IAH-Pirbright, on tests for detection of infection in vaccinated cattle. The study involved samples obtained from 20 “O” Manisa vaccinated and 5 unvaccinated cattle. These were challenged at 3 weeks post vaccination, by 5 day contact with 5 “O” UKG 2001 needle-

challenged donor cattle. Unvaccinated and donor cattle were removed after 5 days. All challenged cattle were kept for 28 days and 15 challenged cattle were kept for approximately 6 months. A summary of the results is presented in Table 1. All of the unvaccinated cattle developed FMD. Although none of the vaccinated cattle showed any clinical signs of FMD, nor any FMDV viraemia, they could be divided into three categories on the basis of virological tests carried out on post-challenge oropharyngeal fluid samples. One group of 5 animals appeared not to have been infected (Group 1). A second group of 6 animals showed a transient infection persisting up to 12 days post challenge (Group 2). A third group of 9 animals became persistently infected with FMDV (Group 3). Blood samples collected from all of these animals for up to 91 days post challenge were tested by the Ceditest FMDV-NS ELISA, the UBI FMDV 3B peptide NSP ELISA and the Bommeli CHEKIT-FMD-3ABC ELISA. A fourth serological NSP test used was an indirect ELISA with a 2B peptide (Inoue, personal communication). Finally, saliva samples collected at the same time points as the blood sampling were analysed by ELISA for FMDV structural protein-specific IgA antibody.

Most of the assays were effective at detecting infection in the unvaccinated cattle. One animal was largely missed by the UBI test whilst salivary IgA levels waned to undetectable levels in some of these cattle within 3 weeks of infection. Most of the samples collected from the cattle in Group 1 scored negative in most of the tests. No reactivity was seen at all with the UBI and Cedi Diagnostics tests, whilst the most reactivity was seen with the IgA saliva test. Only one animal in Group 2 was kept beyond 28 days post challenge. None of the samples collected from this group were positive in the UBI or 2B tests, whereas some samples were positive in the Bommeli and Cedi Diagnostics tests. Many samples were positive by the IgA saliva test with a peak of reactivity at 16 days post infection and a subsequent decline thereafter. All 9 animals in Group 3 (the important persistently infected group) were monitored beyond 91 days post challenge. The order of sensitivity of detection of infection in these animals was:

1. IgA Saliva (beyond 21 days post challenge, detected 8/9 animals most of the time).
2. Cedi Diagnostics test (beyond 28 days post challenge, detected 7/9 animals)
3. 2B Peptide ELISA (maximal detection of 6/9 animals at 42-63 days post challenge)
4. UBI ELISA (maximal detection of 6/9 animals at 63 and 70 days post challenge)
5. Bommeli ELISA (maximal detection of 3/9 animals).

Different tests missed different animals and no one test detected all of them.

It is impossible to recreate all possible scenarios of FMD exposure experimentally. These results demonstrate that there is considerable variation between vaccinated cattle in their responses to a high level of contact challenge. Such differences are likely also to be seen under field conditions. Further experiments to mimic field exposure are also needed in sheep and pigs. The findings confirm that 100% detection of persistently infected animals is difficult to achieve and that there are considerable differences in the performance of different assays. The IgA test and the 2B peptide test both merit further development.

Acknowledgement

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References

Anonymous (2003). Council directive on community measures for the control of FMD repealing Directive 85/511/EEC and Decisions 89/531/EEC and 91/665/EEC and amending Directive 92/46/EEC.

Bergmann et al. (2000). Improvement of a serodiagnostic strategy for foot-and-mouth disease virus surveillance in cattle under systematic vaccination: a combined system of an indirect ELISA-3ABC with an enzyme-linked immunoelectrotransfer blot assay. *Arch Virol.* 145, 473-89.

Chung et al (2002). Differentiation of foot-and-mouth disease virus-infected from vaccinated pigs by enzyme-linked immunosorbent assay using nonstructural protein 3AB as the antigen and application to an eradication program. *J Clin Microbiol.* 40, 2843-8.

Crowther (2001). Experiences of IAEA on the use of non-structural antigens of FMD virus to assess antibodies in vaccinated and infected livestock. Proceedings of EUFMD Research Group Meeting, Island of Moen, Denmark, pp98-102.

Grunmach et al (2001). Bayer: results achieved so far with the test for antibodies against NSP's of FMDV. Island of Moen, Denmark, pp84-87.

Haas & Sorensen (2002). Comparison of ELISAs for the differentiation of infection from vaccination by detection of antibodies to NSP 3ABC of FMDV. Proceedings of EUFMD Research Group Meeting, Izmir, pp248-256.

Jacobson (1998). Validation of serological assays for diagnosis of infectious diseases. *Rev. sci. tech. Off. Int. Epiz.*, 17, 469-486.

Liu et al. (2002). Synthetic peptide-based serosurveillance and vaccine system for FMD. Proceedings of EUFMD Research Group Meeting, Izmir, pp303-321.

Moonen et al. (2000). Diagnosis of persisting infections of FMDV in cattle: IgA ELISA, virus isolation and RT-PCR. Proceedings of EUFMD Research Group Meeting, Borovets pp188-9.

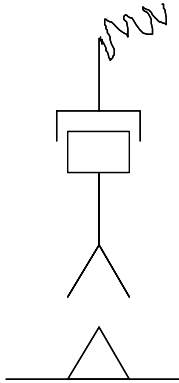
OIE (2000). OIE Manual of Standards for Diagnostic Tests and Vaccines. 4th Edition. Paris, France.

OIE (2002). Report of the meeting of the OIE ad hoc group on evaluation of nonstructural protein tests for foot-and-mouth disease diagnosis. Paris, 2-4 October 2002.

Schalch et al. (2002). Recently generated data with the CHEKIT-FMD-3ABC ELISA kit and methods to monitor the operational performance of a 3ABC ELISA. Proceedings of EUFMD Research Group Meeting, Izmir, pp283- 302.

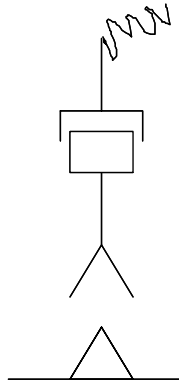
Fig 1. Schematic comparison of three FMD NSP ELISA kit methods, with key differences between tests underlined.

Bommeli
CHEKIT-FMD-3ABC



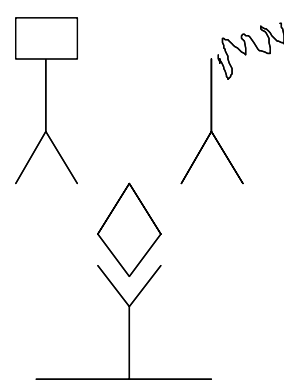
Indirect ELISA
Species specific kits
Serum predilution step
Takes 3 hrs
+ve / -ve control serum
Result rel. to +ve control
Ambiguous range

UBI
FMDV NSP ELISA



Indirect ELISA
Species specific kits
Serum predilution step
Takes 2.5 hrs
+ve / -ve control serum
Result rel. to +ve control
No ambiguous range

Cedi Diagnostics
Ceditest FMDV-NS



Blocking ELISA
One kit for all species
No serum predilution step
Takes overnight or 4 hrs
+ve / -ve control serum
Result rel. to +ve control
No ambiguous range

Table 1. Summary results for vaccine challenge study in which vaccinated or unvaccinated cattle were challenged by contact with FMDV inoculated donor cattle

- All 5 unvaccinated controls showed severe clinical disease (more severe than after needle challenge)
 - Virus was isolated from oropharyngeal fluid samples and blood
 - 2 possible persistently infected animals (positive by RT-PCR only at 28 or 42 dpc)
 - 3 animals retained for several months post challenge
 - Consistent responses in most tests for infection

- All 20 vaccinates clinically protected
 - No viraemias detected
 - 5 animals: no oropharyngeal virus isolated post challenge
 - 6 animals: probang virus isolated sporadically up to 12 dpc
 - 9 animals became persistently infected
 - 12 animals retained for several months post challenge
 - Considerable variation in responses to different tests for infection