Validation and application of non-structural protein antibody tests in post-vaccination serosurveillance to demonstrate foot-and-mouth disease freedom after use of an emergency vaccinate-to-live policy

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Conclusions

1. Available tests for detecting FMD infection, including “carrier” animals in vaccinated cattle have been validated.

2. They are not yet fully validated for use in pigs and sheep but work to achieve this is in progress.

3. The tests will detect infection in herds where there has been significant cattle-to-cattle spread, but confirmatory tests are needed to deal with problems of false positive results and to enable small numbers of carrier cattle to be detected with confidence.

4. Sampling and testing regimes and methods of test result interpretation that are best able to determine if infection is present at both the herd and population level are being finalised.

5. No testing regime will guarantee disease freedom (i.e. identify all infected animals) and the required confidence with which freedom from infection must be demonstrated remains to be agreed and incorporated into international guidelines.

Introduction

The new EU Directive on foot-and-mouth disease (FMD) makes provision for the use of the so-called “vaccinate-to-live” policy for the control of FMD in Europe. However, there are uncertainties about how effective this would be in different situations and about how readily FMD-free status could be regained thereafter. According to this approach, spread of the FMD virus from future outbreaks could be controlled by a limited period of “emergency” vaccination of surrounding herds, reducing the need for large-scale pre-emptive culling of at-risk animals. Vaccinated animals may still become subclinically infected with FMD virus following challenge exposure and in the case of ruminants, this infection may persist. In order to rapidly regain the most favoured trading status of FMD-free without vaccination, current trade rules require that all vaccinated animals that are infected should be detected and either killed and destroyed or else slaughtered for consumption under controlled conditions. This can be attempted by testing vaccinated animals for the presence of antibodies to certain non-structural proteins (NSP) of FMD virus, which are induced by FMD infection, but not by vaccination with purified vaccines. The numbers of herds and animals to be sampled and tested to be confident that infection has not been missed will depend upon the expected prevalence of subclinical infection amongst and within herds. This in turn will depend upon the manner in which infection is spread and on how vaccination is applied. The sensitivity of the tests used and the size of the herds will also influence the numbers of samples required to be collected and tested and the certainty of the interpretation. This paper summarises the
issues and uncertainties over use of NSP testing in support of a vaccinate-to-live policy and the results of coordinated efforts to quantify the specificity and sensitivity of NSP ELISAs. It also considers the ways in which the tests can be used and interpreted and the effect that this will have on the confidence with which freedom from infection can be demonstrated.

**Diagnostic performance of tests for antibodies to FMDV non-structural proteins**

Validation of NSP tests for use to support a vaccinate-to-live policy in Europe has been delayed whilst new tests were developed and because it requires large panels of sera representative of different livestock species that have been vaccinated or vaccinated-and-infected with different serotypes of FMDV. Furthermore, it should be known when the animals have been infected and whether or not they showed signs of disease, supported extensive virus replication and became carriers. To establish test specificity, sera should preferably come from animals that have been vaccinated with equivalent vaccines to those likely to be used in Europe and sampled at least a month after vaccination. To establish sensitivity, sera should come from animals that have been vaccinated and then infected, preferably without showing clinical signs but known to have become carriers.

To address this difficulty, a consortium of laboratories from Europe, Israel, Turkey and South America were assembled under the auspices of the EUFMD and the EU FMD Improcon project of the European Sixth Framework Research Programme. Each contributed sera from the field and/or experimental animals enabling a panel of several thousand samples to be assembled for testing at a workshop in Brescia during May 2004. A panel of field sera from Zimbabwe was later added and tested. All of the sera were tested in parallel by six different methods, including four commercial assays, one “in-house” test and the OIE index method from PANAFTOSA. The analytical sensitivity of the tests was also compared using reference serum titrations.

An analysis of the results of the workshop will be presented. The main conclusions are that:

1. There is sufficient data to validate the tests for use in cattle, but more samples are needed to establish the sensitivity in sheep and pigs.
2. The specificity of the tests is not affected by a single vaccination with European vaccines. In cattle it ranges from 97% to 98.5% in the different tests, but improves after retesting false-positive samples and may then approach 99%. False-positivity is not observed simultaneously in different assays, i.e. each sample reacting as false-positive in a test is usually negative in all the others.
3. The extent of virus replication correlates with the production of NSP antibodies so that animals that are significant shedders or carriers are more likely to be detected.
4. The sensitivity of all the tests approach 100%, in non-vaccinated and infected cattle collected up to 100 days after experimental infection.
5. In contrast, the sensitivity for detecting vaccinated carriers (cattle in which persistent infection between 28 and 100 days was demonstrated) varies significantly between tests, ranging from 66% for the less sensitive up to 92% for the most sensitive test.
6. Considerable differences in analytical sensitivity of the tests were observed for detection of antibodies in serially diluted reference sera, but did not correlate with the relative diagnostic sensitivity of the tests.
7. The three assays with highest diagnostic sensitivity on experimental sera also show higher detection rates and concordance when applied to field panels. Detection rates recorded for 465 serum samples from vaccinated cattle in infected herds in Israel varied from 15% to 25%. Detection rates recorded for 402 serum samples from five infected herds in Zimbabwe with variable vaccination status ranged from 48% to 67%, suggesting a higher degree of viral circulation. These results from field samples
do not allow one to estimate absolute sensitivity values, but they provide crude estimates of antibody prevalence in different field situations.

8. Overall, NSP tests are at present the most sensitive tool to detect carriers in a single sample. Tests results for infected cattle are highly correlated among all six tests, as proven by the analysis of the conditional dependence; however the extensive comparison enabled us to graduate the sensitivities of tests available in Europe and compare them to the sensitivity of the OIE index test from PANAFTOSA.

Issues of uncertainty/complexity

1. Disease freedom needs to be established at the level of the whole population and not just at that of the individual herds or animals. The relationship between determining the statistical probability of the population being infected or not and the sensitivity and specificity of tests for detecting individual infected animals is complex.

2. Is it important to be able to detect a given prevalence of infection in vaccinated herds or to be able to detect a minimum absolute number of infected animals within each herd?

3. What proportions of herds or flocks will become subclinically infected despite vaccination?

4. Within herds or flocks that become subclinically infected despite vaccination, what proportion of animals will be infected?

5. How significant are subclinically infected vaccinates for the onward spread of disease, especially once more than a month has elapsed (the so-called “carrier” debate)? Should special attention be given to bulls? No system of testing can guarantee to disclose all infected animals so a consensus is needed on what represents an acceptable risk.

6. Is the purpose of testing to detect and cull previously infected animals and herds or only to detect and cull animals that are still carrying virus, i.e. either acutely infected (virus circulation) or persistently infected (carriers)? Unlike EU regulations, OIE trade rules do not seem to require that action is taken against herds where virus has circulated but in which infection is no longer present.

7. How sensitive and specific are NSP ELISAs for the detection of infection in vaccinated animals and how is this affected by a number of factors including?
   - Species of animal and serotype of FMDV
   - Vaccine dose and repetition
   - Route and weight of challenge exposure and interval between vaccination and challenge
   - Extent of virus replication and persistence
   - Interval between challenge exposure and sampling

8. Sensitivity for detecting infected animals can be increased by herd-based approaches but this leads to difficulties with
   - Small herds in which a low design prevalence for detection of infected animals may not be possible to realise.
   - Specificity, since testing large herds may result in a high proportion being scored as false positive. There is therefore a requirement for confirmatory tests.
9. Which are the best tests, what system of sampling, testing, retesting and further investigation will be optimal, what level of confidence will this give and how much testing and retesting will be required?

**Effect of test sensitivity and specificity on limits of detecting carrier cattle**

Factors to be considered in addition to individual test and overall test regime performance are the size of the herds, the number of animals sampled, the minimum percentage or absolute number of infected animals to be detected and the confidence level required.

With a test of 100% specificity, confirmatory testing is not required. If combined with 95% sensitivity, it is possible to detect a single infected animal with 95% confidence, in any size of herd, but only if every animal is sampled. Even so, it is to be anticipated that 5% of carriers will be missed. The available NSP tests do not provide this level of sensitivity and specificity although their performance may be improved by additional testing with or without resampling and by more sophisticated interpretation, such as weighting of strong positive or clustered test results. Work is in progress to determine the optimal testing regimen possible. Thereafter, the level of infection that can be detected at specified levels of confidence can be established as well as the likely extent of follow-up investigations required.

Finally, herd level sensitivity and specificity must be related to the confidence with which the overall status of the population in question can be determined.