## CHAPTER 12

# POST-OUTBREAK SURVEILLANCE

## INTRODUCTION

International trade cannot resume after an outbreak of FMD until the country involved has proven to the satisfaction of the European Commission and other Member States of the EU, and the Office International des Epizooties (OIE) that it is free of the disease. This section describes the proofs required and explains how they are to be obtained through clinical and serological surveillance in the infected area(s) and the rest of the country.

## CONTENTS

## 1. REASONS FOR CARRYING OUT POST-OUTBREAK SURVEILLANCE

**1.1** **To meet EU requirements for surveillance within the Protection and Surveillance Zones.**

These requirements are laid down in Articles 36 and 44 of the draft Commission proposal to amend Directive 85/511/EEC [Document COM (2002) 736 final] and are summarised in Annex 1 – Zone Clearance Protocol, Chapter 17, **Creation of control zones, census and surveillance**.

**1.2** To regain "FMD free status where vaccination is not practised" and thereby allow a resumption of international trade under OIE rules.

## 2. REGAINING OIE RECOGNITION OF FMD FREE STATUS WITHOUT VACCINATION

**2.1** OIE rules require either:

- 3 months freedom after the last case, if stamping out and serological surveillance are employed, or
- 3 months freedom after the slaughter of the last vaccinated animal if stamping-out, serological surveillance and emergency vaccination are applied, or
- 6 months after the last case or the last vaccination (according to the event that occurs the latest), where a stamping-out policy, emergency vaccination not followed by the slaughtering of all vaccinated animals, and serological surveillance are applied, provided that a serological survey based on the detection of antibodies to nonstructural proteins of FMDV demonstrates the absence of infection in the remaining vaccinated population

**2.2** An OIE "Draft guide to the establishment or the regaining of recognition for a foot and mouth disease free zone or country" is currently available in draft form (see **Annex 1**). This Guide gives the principles involved in designing a post-outbreak serological survey and will be used in the event of an FMD outbreak.

## 3. SURVEILLANCE CARRIED OUT IN 2001

**3.1** Following FMD in 2001, a national serological survey of sheep was carried out. This survey was based on the OIE Guide in **Annex 1**.

**3.2** The design of the survey is given in **Annex 2**.

**3.3** An example of instructions to DVOs is given in **Annex 3**.

**3.4** See Chapter 2, **Veterinary Laboratory Service** for further details.

# ANNEX 1

# OIE DRAFT GUIDE TO THE ESTABLISHMENT OR THE REGAINING OF RECOGNITION FOR A FOOT AND MOUTH DISEASE FREE ZONE OR COUNTRY

The following are foot and mouth (FMD) surveillance guidelines for countries or zones applying to the OIE for FMD freedom without vaccination, or for countries or zones applying to the OIE for FMD freedom with vaccination. These guidelines are not intended to exclude other verification strategies, but if an alternative strategy is used, it is essential that it is statistically defensible.

Surveillance for FMD may be part of a continuing disease surveillance programme involving regular checks on livestock at all stages of the production chain up to slaughter or export, or it may be a specific programme designed to establish that FMD infection is absent from the national herd in the whole territory or part of it (free zone). The OIE *International Animal Health Code* recognises countries or zones being free of FMD infection, either with or without vaccination.

## General Conditions

A surveillance system for FMD must be supported by an efficient and adequately funded state veterinary service with expertise on the epidemiology of FMD, and access to a diagnostic laboratory capable of undertaking FMD diagnosis and serology and a farming community committed to the recognition and reporting of FMD. Training of veterinarians, whether state or private practitioner, and animal health auxiliaries in the clinical recognition of FMD and the collection and dispatch of samples is essential, together with an information programme directed at farmers and other animal workers on the importance of early notification of disease outbreaks. There must be in place a procedure for the rapid transport of samples to the laboratory, and access through the laboratory for onward dispatch of samples to the national, regional or world reference laboratory.

Passive surveillance is an ongoing programme that should be used by all Veterinary Services to monitor for the appearance of disease in the national livestock populations. Active surveillance is specific in respect of confirmation of the suspect presence of a particular disease and quantification of its prevalence or to demonstrate freedom from a disease/infection for a geographically defined area.

An FMD surveillance programme must:

1) Respond to observations and reports made by the public, and from state and private veterinarians, and in particular the farmer and animal health workers who have day-to-day contact with the national herds and flocks (passive surveillance). Whether or not FMD is already a notifiable disease, legally obliging immediate notification, farmers must be encouraged to report promptly any clinical disease resembling FMD. They must be supported by government information programmes and the state veterinary service directly or through private veterinarians. All reported suspect cases of FMD must be investigated within 24 hours, and, if still considered suspect, samples must be taken and submitted to the national laboratory by rapid transport. This requires that sampling kits, drugs to sedate animals from which samples are being taken, transport and communications and the wherewithal for the decontamination of equipment and clothing of those involved in disease investigation are made available at all times. Both state

and private veterinarians who may be involved with investigating suspect outbreaks of FMD must be familiar with the clinical signs and epidemiology of FMD, and have been trained in sample collection. They should also have access to relevant information on the current FMD status of their own and neighbouring countries, and any particular risk factors, and be able to call for additional advice and help from a specialized government FMD epidemiological team. Laboratory results must be sent as soon as possible to the relevant person in the state Veterinary Service, and to the veterinarian submitting the sample, to encourage future co-operation.

The level of this surveillance can be assessed by the number of farmer and other reports received by the state Veterinary Service and the number of investigations carried out, together with the results of the investigations.

When relevant, include regular and frequent clinical inspection and serological testing of high risk groups of animals, such as those adjacent to an FMD infected country or zone (for example, bordering a game park in which there is infected wildlife).

These general conditions are required for all Member Countries submitting their annual request for re-confirmation of FMD free status. Evidence of an enhanced surveillance programme is required from Member countries applying for the first time for recognition of freedom from FMD with or without vaccination.

## Countries or zones applying for freedom from FMD virus infection where vaccination is not practised

In addition to the general conditions, a Member Country applying for freedom from foot and mouth disease virus (FMDV) infection must show evidence of an active surveillance programme in which the FMD susceptible population undergoes regular clinical examination, and a statistically significant sample of this population is tested for evidence of FMDV infection. This requires the support of a national or other reference laboratory able to undertake serology for FMDV antibody using an OIE accepted test; as described in the most recent edition of the OIE *Manual of Standards for Diagnostic Tests and Vaccines*, or as updated by a resolution from the International Committee of the OIE between editions of the Manual.

In general, the target population of the random sample survey will consist of the susceptible species within the country or zone to be declared free from disease. Countries wishing to show freedom from FMD in which a pig-specific strain of virus had been prevalent should concentrate on sampling the national pig population. In countries in which an African buffalo population is present, this should also be sampled if included in the proposed FMDV infection-free area. The inclusion of other species of wildlife ruminants in a survey is unnecessary unless there is reason to believe that they are involved in the epidemiology of FMD in the region.

The objective of the random sample design is to keep the volume of surveillance work to the minimum consistent with demonstrating the absence of infection at the required level of statistical confidence. The samples must be selected on a random basis during each of the consecutive sampling campaigns; the frequency of the sampling will depend on the epidemiological situation, but should be at least once during the year preceding the application. It must be ensured that every sampling unit has an equal selection probability. The selection of individual sampling units should not affect the probability of selecting any other sampling unit. It must be emphasized that a random selection of the sampling units is absolutely essential; otherwise the required level of statistical confidence cannot be achieved.

In order to provide representative information on the infection status of the target population, the random sample survey ought to be completed within the shortest possible period of time.
The population may be divided into sections (strata) with similar epidemiological conditions within each stratum. Stratification implies that a suitable system of separating the target population into a series of sections or strata from which random samples can be drawn has to be developed. A stratum should be a subpopulation of the total population that is raised using a similar production and husbandry system under similar ecological conditions within geographical or administrative areas (provinces, states, etc.) with a similar risk of infection. Which of these stratification criteria will be most appropriate will depend on the conditions prevailing in the individual country.

During the process of stratification the following two conditions have to be met:

- All sampling units (village, flock or herd depending on farming system) within a particular stratum can be accessed during the survey and have an equal chance of being selected.

- An individual sampling unit is included in only one stratum.

The total number of strata required will depend on the country or zone concerned and additional strata or an increased level of sampling may be applied to areas within a country or zone considered to be at higher risk of FMDV infection. Care should be taken that the number of strata does not exceed the capacity of the field and laboratory service as the required number of random samples will have to be collected from each of the strata. The number of samples is determined, to a considerable extent, by the number of strata. Hence the number of strata should be kept to a minimum but reflect major epidemiological differences. Further detail may be obtained from suitable epidemiological texts (see references).

If a Member Country wishes to declare a specific zone within the country free from FMDV infection this must be taken into consideration in the stratification process. The basis for the sampling process would then be the population within each zone.

The objective of the random sample survey is the detection of clinical or serological evidence of FMD within the population if it is present at a predetermined prevalence. The probability of detecting evidence of FMD or FMD infection in a given sample of animals depends on the prevalence of FMDV infection in the population and the size of the sample. Hence, the sample size and expected disease prevalence determine the level of confidence of the result of the survey. The lower the prevalence the larger the sample size has to be in order to achieve a given confidence in the outcome of the survey. It is recommended that a sampling strategy be used to give a 95% probability of detecting evidence of FMD or FMD infection if it is present in 1% of the primary sampling units. In other words, if at least 1% of herds/flocks are infected with FMD virus, the sample size has to be large enough to give a 95% chance that at least one infected herd/flock will be detected through examination of the random sample of herds/flock.

Clinical surveillance aims at the detection of clinical signs of FMD by close inspection of the mouth, feet and udder of a randomly selected sample. It is essential that all animals within the selected primary sampling unit are examined for signs of FMD. Any herd/flock where suspicious animals are detected is classified as infected until other evidence is produced.

Serological surveillance aims at the detection of antibodies against FMDV. A positive reaction to an FMDV antibody detection test can have four possible causes:

- natural infection with FMDV.
- vaccination.
- maternal antibodies from an immune dam (antibody reaction is usually only up to six months of age in cattle, however, in some individuals and in buffalo calves, maternal antibody can be detected for longer);
- Non-specific reactions to some other unrelated antigen.

Thus antibodies detected in animals (other than African buffalo) over six months of age and born after a country or region has ceased vaccination should be in response to natural infection and be indicative of circulating virus. This group of animals will be considered eligible as secondary sample units for the purpose of serological surveillance. It may be possible to use serum collected for other survey purposes, but the objective of a statistically valid random survey for the specific presence of FMDV should not be compromised.

If vaccination cannot be excluded as the cause of positive serology, additional testing for the presence of antibodies to the non-structural protein (NSP) of FMDV could indicate the previous presence of live FMDV.

It is unlikely to find only one or two seroconverted animals in an infected herd/flock. For this reason and for practical as well as economic reasons it is considered acceptable to include only a random sample of animals from each primary sampling unit in the serological surveillance. The sample size has to achieve a 95% probability of detecting seroconverted animals. If a herd is infected after the cessation of vaccination, it is expected that the serological prevalence will exceed the 20% level.

FMDV persists in the pharyngeal region of recovered ruminants for up to 3 years in cattle and nine months in sheep, and therefore oro-pharyngical (OP) fluid sampling is an additional valuable tool in surveillance for FMDV. OP samples should be collected from herds and flocks selected by positive serology. The collection of OP samples will depend on the availability of collection equipment (e.g. probang), facilities for storing the OP material until testing, and access to a laboratory able to work with live FMDV. Sheep can also be sampled by collecting OP fluid, and a similar sampling strategy can be applied, bearing in mind that the carrier state is shorter in this species.

Staff collecting OP samples should be given specific training on the techniques for the collection, transport and storage of OP fluid. It is essential that the OP fluid is placed in a neutral buffer and immediately frozen in or over liquid nitrogen or solid CO2 after collection, and kept in this state until thawed in the diagnostic laboratory and placed on susceptible tissue culture (see OIE *Manual of Standards for Diagnostic Tests and Vaccines*).

It is preferable to stratify the sampling frame to reflect the possibility of FMD being present up to three years previously. OP samples should be collected from each group of yearlings, two-year-old and three-year-old cattle/sheep in the selected herds and flocks.

If returning to a suspect herd/flock, it is recommended that a sampling size for each age stratum (yearling, one year, two year) should be used as indicated above.

The results of the random sample survey will serve as evidence to both to the national authorities and to the OIE that no FMDV infection is present in the country or zone. It is therefore essential that the random sample survey can be audited through clear documentation and the presence of complete records.

## Countries or zones applying for freedom from FMD where vaccination is practised

In addition to the general conditions, a Member Country applying for recognition of freedom from FMD with vaccination must show evidence of an active surveillance programme for clinical disease.

## Countries or zones re-applying for freedom from FMD virus (where vaccination is or is not practised) following an outbreak

In addition to the general conditions, a Member Country re-applying for freedom from FMDV infection or from freedom from FMD were vaccination is practised must show evidence of an active surveillance programme. Four strategies are recognised by OIE in a programme to eradicate FMDV infection following an outbreak:

1) slaughter of all clinically affected and in-contact susceptible animals,

2) slaughter of all clinically affected and in-contact susceptible animals and vaccination of at risk animals, and subsequent slaughter of vaccinated animals,

3) slaughter of all clinically affected and in-contact susceptible animals and vaccination of at risk animals, without subsequent slaughter of all vaccinated animals,

4) vaccination used without slaughter of affected animals or subsequent slaughter of vaccinated animals.

In all circumstances, a Member Country re-applying for freedom from FMDV infection or FMD with vaccination must report the results of an active surveillance programme in which the FMD susceptible population undergoes regular clinical examination. In addition a statistically significant sample, targeted at the susceptible population at risk during the outbreak, is tested for evidence of FMDV infection. The procedures to follow are described above, but when a Member Country has used vaccination to help control the outbreak, and not subsequently slaughtered the vaccinated animals, it may be necessary, under certain circumstances, to test a high proportion of the vaccinated animals using a test for NSP antibodies in order to provide convincing evidence that the FMDV has been eliminated. The time required before an application can be made to the OIE is specified in Article 2.1.1.6 of the OIE Code, and depends on the control strategy employed.

The use and interpretation of serological tests. (see also Fig 1)

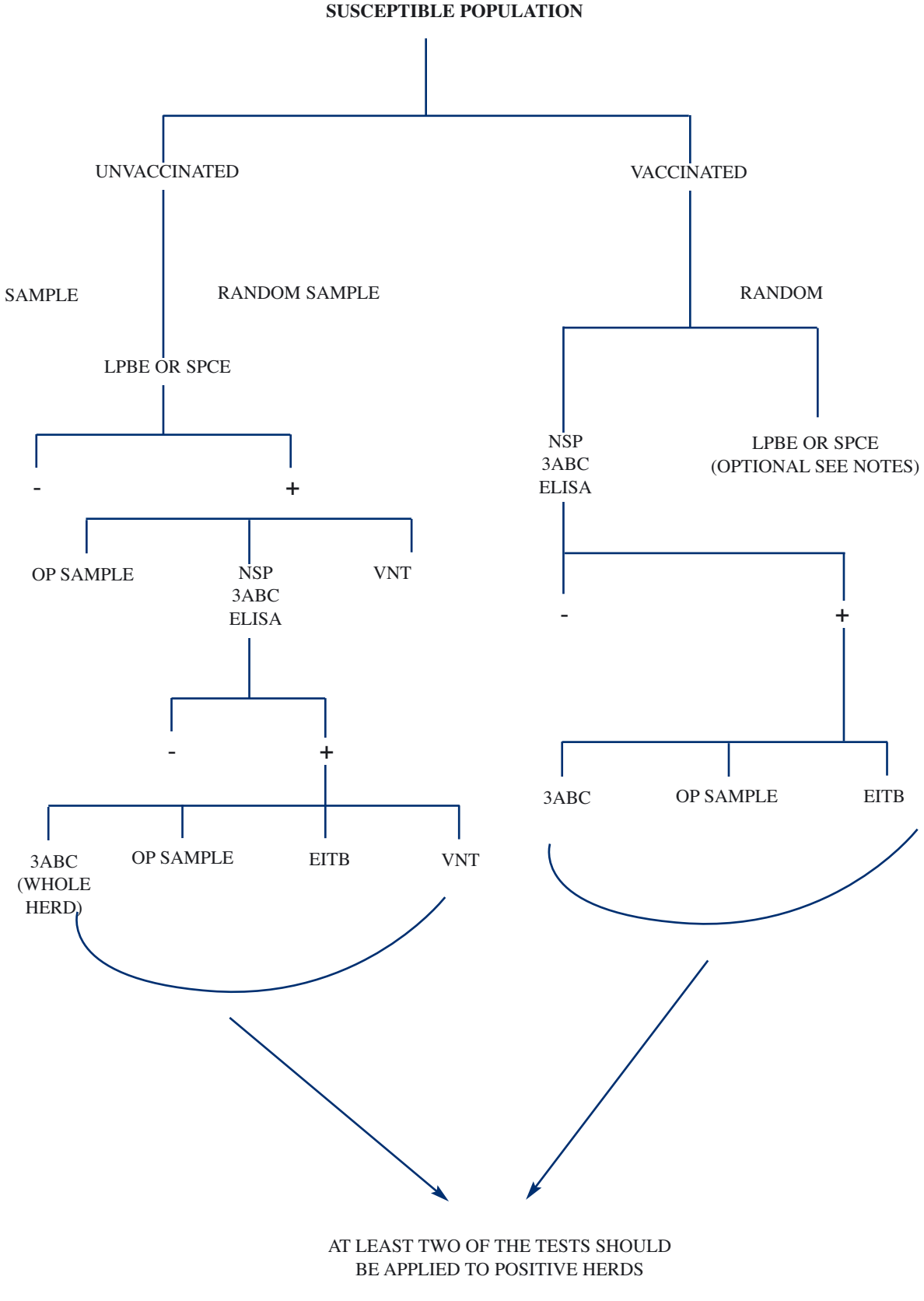
The recommended serological tests for FMD surveillance are described in the *Manual of Standards for Diagnostic Tests and Vaccines* (O.I.E. 2000). In unvaccinated populations, the screening can be carried out using the liquid phase blocking ELISA (LPBE). This is a very sensitive test approaching 100% sensitivity, but it can have a specificity in cattle as low as 95%, and will therefore give up to 5% false positive results using the titre of above 40 as positive. Because the objective of the survey is to discover evidence of infection if it is present, it is acceptable for the purposes of the survey to raise the cut-off value for negative/positive sera. This may still result in false positive results, and these sera should be re-tested by the virus neutralisation test (VNT), in which a titre of 45 or greater is classified as positive. Any animals whose sera are positive by the VNT should be re-sampled to confirm this status, and if still positive they should be tested for evidence of infection. The remaining animals in the herd/flock should also be tested for the presence of FMDV antibodies, and if found positive, sampled by collection of oesophageal-pharyngeal material using a probang cup. Although not a prescribed test, the solid phase competition ELISA (SPCE) has been shown to have a higher specificity, but similar sensitivity to the LPBE, and may be used in preference to the LPBE.

For serological surveillance in countries or zones in which vaccine is, or has been used, the LPBE or SPCE can still be the test of choice in those FMD susceptible species not included in the vaccination programme. Animals that have been vaccinated will have antibodies to the structural proteins of FMD virus, and some may have antibodies to the NSPs, depending on the number of times they have been vaccinated, and the amount of the NSPs present in the vaccine used. However, animals that have recovered from infection with FMD virus will have high levels of antibody to the NSPs. There are eight NSPs associated with the replication of FMD virus, namely L, 2A, 2B, 2C, 3A, 3B, 3C and 3D, and antibodies can be found to all of these in most recovered animals. Some do not persist for more than a few months, and some animals may fail to produce detectable levels to all of them. ELISA tests have been developed to detect 2C and 3ABC antibodies, the former being detectable for up to one year after infection, and the latter for up to two years. A western blot technique (EITB) has also been used to detect the NSP antibodies to 2C, 3ABC, 3A, 3B and 3D which is particularly specific and sensitive in identifying animals previously infected. All these tests have been validated in cattle.

A class of animal exists, however, that has been infected with FMD virus and could remain carrying the virus without developing detectable antibodies to the NSPs. These are animals, which have received highly potent vaccine and then have contact with the virus during an outbreak, but because of their level of immunity, suppress viral replication and show no evidence of disease. Because the virus does not significantly replicate in these animals, there is little expression of the NSPs and therefore no development of detectable levels of antibodies. However, on a herd basis there are always less protected animals following vaccination, and if these animals are challenged with the virus, they will produce antibodies to the NSPs, and can develop clinical disease. It is therefore important that the NSP antibody test be interpreted by assessing the level of these antibodies in the sera of a representative sample from the whole herd.

There is the option to use the non-structural antibody test together with the LPBE or SPCE, particularly in areas where vaccination has been used and virus activity is suspected. LPBE titres or SPCE inhibition higher than would be expected from vaccination alone may suggest FMD virus infection and this can be confirmed by testing for the presence of antibodies to the NSPs, and by taking OP samples.

*Fig 1: Schematic representation of laboratory tests for determining evidence of FMD virus infection*



## ABBREVIATIONS

| | |
|---|---|
| ELISA: | enzyme linked immunosorbant assay |
| LPBE: | liquid phase blocking ELISA |
| SPCE : | solid phase competition ELISA |
| VNT: | virus neutralisation test |
| NSP: | non structural protein(s) of FMDV |
| 3ABC: | NSP antibody test |
| EITB: | western blot for NSP antibodies of FMDV |
| OP: | oesophago-pharyngeal sample |

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# ANNEX 2

# PROGRAMME TO ESTABLISH THE ABSENCE OF FMD IN THE NATIONAL SHEEP FLOCK IN IRELAND, FOLLOWING A SINGLE OUTBREAK OF FMD IN SHEEP IN MARCH 2001

## 1.1 Introduction

This document concerns the design of a survey to establish freedom of the national sheep flock from FMD in accordance with guidelines provided by the OIE. The survey design was drawn up based on the following documentation.

a) OIE 2001. *Guide to the Establishment and Maintenance of FMD Free Zone or Country*.

b) Cannon, R.M., Roe, R.T. 1982. *Livestock disease surveys. A Field Manual for Veterinarians*. Bureau of Rural Science, Department of Primary Industry. Australian Government Publishing Service, Canberra.

c) James, A.D., 1998. *Guide to epidemiological surveillance for rinderpest*. Rev. sci. tech. Off. int. Epiz., 1998, 17 (3), 796-809.

d) Martin, S.W., Meek, A.H., Willeberg, P., 1987. *Veterinary Epidemiology. Principles and Methods*.

## 1.2 General considerations

The objectives of the survey design are two fold. The first objective is to demonstrate the absence of FMD in Ireland to the level of statistical confidence stipulated in the OIE guidelines. To achieve this objective it is necessary to select sampling units randomly to allow quantification of confidence in the results, as well as helping to eliminate bias in selection of sampling units.

The second objective is to test as many flocks as possible for FMD, in the most efficient and effective way given a fixed resource in terms of the numbers of samples which can be analysed at the Central Veterinary Laboratory (CVL) Abbotstown. For the purposes of this survey a ceiling of 200,000 tests was set as the maximum number of tests available to achieve the objectives stated above.

In view of the fact that Ireland has a long history of freedom from FMD and only one case has been recorded in the March outbreak, the number of flocks with potentially seroconverted sheep is likely to be very small. Therefore, any survey that concentrates on sub-populations which may have been exposed to higher levels of risk is more likely to detect disease if present. For this reason and to ensure that strata remain mutually exclusive and exhaustive, it was decided to stratify the national sheep flock or target population into 2 strata. Stratum 1 contains flocks with a tradition of unstable sheep populations, which are more likely to have received sheep from the UK or Northern Ireland prior to the closure of the border. Stratum 2 contains flocks in areas with predominantly highland populations which tend to be more stable and thus would not have been exposed to the same level of risk.

In Ireland, individual flocks can be subdivided over several different land parcels subject to various different levels of mixing. Thus the design of this survey takes into account the fact that the target population in each stratum is divided into various levels of aggregation or hierarchical units; with primary units consisting of sheep flocks; secondary units consisting of subpopulations within sheep flocks (so called epidemiological units) and tertiary units consisting of individual sheep within epidemiological units. The definition of tertiary units was further refined by a decision to exclude unweaned lambs from the survey, as it is improbable that unweaned lambs could be seropositive without the ewes also being seropositive.

Although the number of sheep flocks potentially exposed to the FMD virus in Ireland is likely to be very small, it was unlikely if a flock had been exposed that only 1 or 2 animals would have seroconverted. Therefore in an effort to maximise efficiency while maintaining effectiveness, it was decided that only a proportion of sheep in each epidemiological unit would be sampled. However, this approach presents a problem with regard to the calculation of the required sample size for primary units since conventional sample size formulas assume that all individuals in the primary unit are sampled or in other words there is no possibility for sampling error within primary units. Though on one hand it could be argued that as FMD is such an infectious disease, it is most unlikely that a mistake with regard to the status of an epidemiological unit could be made provided a sufficient proportion was tested, it was decided for statistical validity to follow the approach laid down by Dr James (James, 1998) in his paper 'Guide to epidemiological surveillance for rinderpest'. Following this approach, the concept of ***detectable prevalence*** was used in this survey to calculate the number of primary units, which must be sampled.

The final issue, which must be considered, is the timescale for the completion of this survey. Considering, seroprevalence is unlikely to remain constant over time, it is essential to ensure that the survey is completed in the shortest time possible in order to obtain a clear picture of the status of the population at a point in time.

## 1.3 Sampling design

### i) Stratification and sample size considerations

To facilitate stratification, the 26 counties in Ireland were divided up into 28 administrative units as follows.

| STRATUM 1 | STRATUM 2 |
|---|---|
| CARLOW | CLARE |
| CAVAN | CORK |
| EAST DONEGAL | WEST DONEGAL |
| DUBLIN | GALWAY |
| KILDARE | KERRY |
| KILKENNY | LIMERICK |
| LAOIS | MAYO |
| LEITRIM | SLIGO |
| LONGFORD | TIPPERARY SOUTH |
| LOUTH | WATERFORD |
| MEATH | |
| MONAGHAN | |
| ROSCOMMON | |
| OFFALY | |
| TIPPERARY NORTH | |
| WESTMEATH | |
| WEXFORD | |
| WICKLOW | |

The number of flocks in each stratum was estimated using the data in the National Ewe Premium Database, which contains records for all flocks for which ewe premium was claimed from the European Union. From this database it was calculated that the number of flocks in each stratum was as follows.

| STRATUM 1 | | STRATUM 2 | |
|---|---|---|---|
| **COUNTY** | **EP APPLICANTS** | **COUNTY** | **EP APPLICANTS** |
| CARLOW | 997 | CLARE | 715 |
| CAVAN | 838 | CORK | 1986 |
| EAST DONEGAL | 3,288 | WEST DONEGAL | 1650 |
| DUBLIN | 190 | GALWAY | 5573 |
| KILDARE | 687 | KERRY | 2170 |
| KILKENNY | 856 | LIMERICK | 265 |
| LAOIS | 600 | MAYO | 4611 |
| LEITRIM | 879 | SLIGO | 1414 |
| LONGFORD | 561 | TIPPERARY SOUTH | 666 |
| LOUTH | 450 | WATERFORD | 499 |
| MEATH | 1,226 | | |
| MONAGHAN | 444 | | |
| ROSCOMMON | 2,210 | | |
| OFFALY | 851 | | |
| TIPPERARY NORTH | 675 | | |
| WESTMEATH | 1,018 | | |
| WEXFORD | 1,687 | | |
| WICKLOW | 931 | | |
| **TOTALS** | **18,388** | **TOTALS** | **19,549** |

The average number of animals in a flock was 117 providing an estimated prerequisite sample size of 46 animals in the average flock (Cannon and Roe, 1982). For the purposes of estimating the number of samples available after minimum requirements have been met, it was assumed based on information provided that 90% of flocks in the national sheep flock consist of 1 epidemiological unit and 10% consist of 2 epidemiological units.

From the total number in Stratum 2, the number of flocks which need to be tested in order to be 95% confident of detecting a 1% prevalence can be calculated using FreeCalc® Version 2, which incorporates conventional sample size formulas. Utilising this software, the required sample size in Stratum 2 was 314 flocks requiring an estimated 15,870 animal tests.

The calculation of the number of flocks to be tested in Stratum 1 was a little more complicated as this is the stratum in which the majority of sampling resources were to be concentrated. The minimum number of flocks, which need to be tested to comply with OIE guidelines, was 314 flocks.

However, it was one of the goals of this survey to ensure that as many flocks as possible were tested in this higher risk stratum in order to provide additional safeguards significantly in excess of the standard laid down in the guidelines. Utilising the remaining number of animal tests available (184,130), it was estimated that approximately 3,636 flocks could be tested under the assumption that 90% of flocks consist of 1 epidemiological unit.

**i) Sampling methods**

The design used in this survey is an adaptation of the 3 stage sampling design where sufficient primary sampling units (sheep flocks) are tested to be 95% confident of detecting a 1% prevalence. All secondary units (epidemiological units within flocks) are tested and finally sufficient tertiary sampling units (sheep) are tested to be 95% confident of detecting a prevalence of 5%[(1)].

From this design, the detectable prevalence over all primary units can be obtained by multiplying the predetermined seroprevalence among primary units by the probability of classifying flocks correctly or:

0.01*0.95 = 0.0095

Primary sampling units were selected using a simple random selection from the sampling frame in each stratum. As there is no centralised national database of sheep tag numbers, the selection of tertiary units was left up to sampling teams which were instructed to use a stratified sampling technique to randomly select sheep for testing within each epidemiological unit.

(1) The figure chosen is a very conservative estimate of the likely level of seroprevalence within exposed flocks as OIE recommendations suggest that expected levels of seroprevalence are morelikely to be in the region of 20%. However, this figure was chosen to minimise the possibility of incorrectly classifying a flock

### 1.4 Sampling procedure

In the first stage of the design process, a sampling frame was drawn down from the National Ewe Premium Database. Flocks were assigned to one of 2 strata based on the administrative unit in which the flock was located. Of the 26 counties in Ireland, all flocks in 18 administrative units with predominantly lowland flocks were assigned to Stratum 1. All flocks in the remaining 10 administrative units were assigned to Stratum 2. A list of the sampling frame in each stratum was supplied to the Epidemiological Investigation Unit at UCD's Veterinary College to enable random selection of flocks.

The survey was divided into 2 phases. The first phase involved testing of flocks in Stratum 1 where testing resources were to be concentrated. A simple random sample of 3,636 flocks was chosen from the sampling frame in Stratum 1. Selected flocks were sorted according to administrative area and each list was supplied electronically both to the Superintending Veterinary Inspector in the District Veterinary Office responsible for each administrative area as well as to the personnel responsible for the implementation of FMD Surveillance Database. In addition to the list, a set of instructions, a table of within flock sample sizes and a random number table were supplied to each DVO for distribution to sampling teams. Finally, sufficient FMD Survey Tags were supplied to each DVO to enable the identification of all sampled sheep.

Phase 2 operated in a similar manner. A sample of 314 flocks was randomly chosen from the sampling frame provided. Selected flocks were organised and distributed to administrative units accompanied by the documentation listed above.

# ANNEX 3

## CIRCULAR TO FIELD STAFF FOLLOWING FMD IN 2001

Re: National Serological Survey for Foot & Mouth Disease in Sheep

As you will already have been informed, it is the intention of DAFRD to carry out a National Serological Survey for FMD in sheep. The aim of this survey is twofold:

- To satisfy International Organisations that we are FMD-free
- To satisfy ourselves that FMD did not enter the sheep flock undetected, with animals imported from GB or NI prior to the import ban

## 1. LOCATION

The initial phase of the survey will concentrate on 18 counties and the number of flocks to be sampled in each of those **18 counties** is as follows.

| COUNTY | EP APPLICANTS | % OF TARGET FLOCK POPULATION | NO. TO BE SAMPLED IN EACH COUNTY | WEEKLY FLOCK TARGET |
|---|---|---|---|---|
| CARLOW | 997 | 5.259 | 191 | 24 |
| CAVAN | 838 | 4.505 | 164 | 20 |
| DONEGAL | 3,287 | 17.692 | 643 | 80 |
| DUBLIN | 190 | 1.023 | 37 | 5 |
| KILDARE | 686 | 3.692 | 134 | 17 |
| KILKENNY | 855 | 4.602 | 167 | 21 |
| LAOIS | 599 | 3.224 | 117 | 15 |
| LEITRIM | 879 | 4.731 | 172 | 22 |
| LONGFORD | 561 | 3.020 | 110 | 14 |
| LOUTH | 189 | 1.017 | 37 | 5 |
| MEATH | 1,222 | 6.577 | 239 | 30 |
| MONAGHAN | 444 | 2.390 | 87 | 11 |
| ROSCOMMON | 2,209 | 11.890 | 432 | 54 |
| OFFALY | 848 | 4.564 | 166 | 21 |
| TIPPERARY NORTH | 674 | 3.628 | 132 | 16 |
| WESTMEATH | 1,017 | 5.474 | 199 | 25 |
| WEXFORD | 1,685 | 9.069 | 330 | 41 |
| WICKLOW | 1,420 | 7.643 | 278 | 35 |
| TOTALS | 18,579 | 100 | 3,636 | 455 |

It is intended at some point in the future to extend the survey to include the rest of the country and details regarding this extension will be supplied later.

## 2. TIMING

The survey must commence on **Tuesday 8 May**, and must be completed within 8 weeks. In an effort to pre-test the procedures, only 4 counties Wexford, Roscommon, Donegal and Laois will be asked to take samples in the first week. The remainder of the counties will be asked to commence sampling on **Monday 14 May**.

The minimum number of flocks to be sampled in each county on a weekly basis is set down in the table above. It is important that sampling be evenly distributed over the 8 weeks to ensure that the laboratory throughput is maintained. The intention is to test fresh blood samples only. The target for the first week is for the laboratory to receive no more than 2,000 samples per day. As the survey progresses and provided the system works, it is thought that this limit can be increased to 5,000 per day for subsequent weeks.

## 3. SAMPLING

Sampling must be carried out in accordance with the instructions enclosed. The flocks to be sampled have been randomly selected from lists of flocks applying for ewe premia. The survey is mainly serological, but a clinical inspection should also be carried out in accordance with the protocol attached.

## 4. TAGS

All sheep sampled must be tagged. It is intended to supply all DVOs with specific Allflex tags and taggers for this purpose. The tags which will either be blue or green in colour (to avoid confusion with other scheme tags) will be marked with the word DAFRD and a number between 1 and 200,000. These tags are exclusive to the National Survey, and may not be used for identification purposes or for movement.

Unfortunately, it will not be possible to have these tags ready for the commencement of the survey next week. The 4 counties involved in the survey next week, will be supplied with 2,500 white tags marked with word FMD and numbered from 1 to 10,000 (all that could be done at short notice). The tags will be couriered by SDS and we have been assured that the tags will be in each of the 4 trial DVOs first thing on Tuesday morning. Because these tags are the same colour as those which will be used in the soon to be introduced sheep identification scheme, these tags **must be removed** once the flock has been derestricted. It is expected that the coloured tags will be available in all DVOs on Monday 14 May.

## 5. RESTRICTION

Sampled and tagged animals must be restricted using form **FMD 20**, listing the tag numbers involved. The restriction should remain in place until the results of all samples have been received. Farmers should be informed of the purpose of the restriction i.e. to ensure that in the event of a suspect result, the individual animal may be re-bled in order to clarify the situation. Other animals may leave the farm, providing the requirements of current legislation are met. If WTVIs are involved in the sampling, the FMD 20 should be completed by a VI, and the WTVI should fill in the tag numbers and give the notice to the farmer. The possibility of authorising WTVIs under the Diseases of Animals Act on a temporary basis is being looked at.

## 6. LABORATORY

The blood samples for the National Survey will receive initial processing in the **Brucellosis Laboratory in Cork**. To facilitate the dispatch of samples to Cork and to prevent confusion with brucellosis sampling boxes, special blue DGP UK sampling boxes are being provided to each DVO. The boxes should be marked with a label, which identifies the consigning DVO and a label, which identifies the consignee as the Brucellosis Lab in Cork. Each box will have space for up to 68 blood samples and though this may seem inefficient, for ease of processing a separate box must be used for each flock or epidemiological unit.

The boxes will not contain either needles or blood bottles so brucellosis bottles and needles should be used. To avoid confusion, these dedicated boxes *must not be used for the sampling of sheep flocks which are being tested for reasons other than the National Survye e.g. suspect cases, mart traces and re-bleeds*. Samples other than the National Survey should be sent to **Virology, VRL, Abbotstown** as heretofore. These sample boxes should be wrapped in brown paper and labelled appropriately.

Blood samples must be accompanied by form **FM1** which should be completed and enclosed in the sampling box before sealing. Farmer's need to be advised that results may not be available for up to 10 days. Results in the form of a spreadsheet will be e-mailed to each DVO from Abbotstown every morning. It is essential that in the absence of the SVI, responsibility for checking these results be allocated to an alternative staff member.

## 7. STAFF

Subject to overall workload and other priorities, SVIs should offer this work to VIs at the outset. Saturday working and/or unsocial hours may be used to facilitate the task. Where VI manpower is not sufficient to complete the weekly quota, WTVIs should be engaged. The rate agreed is 1.6 times the Brucellosis rate for cattle. Discussions with the VOA are currently underway in regard to the use of PVPs.

Finally, counties experiencing problems in the performance of the survey should flag them by way of the Regional SSVI as soon as possible. Monitoring of sample numbers and procedures will be carried out by the NDCC on a regular basis throughout the survey to enable adjustments to be made where necessary.

There is no doubt that this survey is a big undertaking which will require a lot of effort if it is to be carried out correctly and within the time-scale. Your co-operation is appreciated.

A Costelloe
Deputy Chief Veterinary Officer