EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C1 - Follow-up and dissemination of scientific opinions

OPINION ON

REQUIREMENTS FOR STATISTICALLY AUTHORITATIVE BSE/TSE SURVEYS

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 29-30 NOVEMBER 2001.

REQUIREMENTS FOR STATISTICALLY AUTHORITATIVE BSE/TSE SURVEYS

MANDATE

1. According to current E.U. legislation, Member States and third countries shall be classified according to their BSE status. The procedure starts with a risk assessment such as the GBR[1], followed by the assessment of certain defined criteria. However, if the Commission finds that the information submitted is insufficient or unclear, it may determine the BSE status on the basis of a full risk analysis. It must include a conclusive statistical survey of the epidemiological situation in the country concerned. The SSC was invited to address, in the light of the current TSE rapid testing possibilities, the following questions:
   - What are the requirements for a conclusive statistical survey to be used as a basis for the classification of a country according to its BSE status?
   - Which sub-populations should be tested and at what sample size? Is it possible to focus the sampling on certain sub-populations only? The sample size should be adapted to practical circumstances, such as possible temporal and geographical variation in challenge.
   - Are there collateral measures that have to be taken to ensure validity of the data in terms of, for example, data collection and sampling and laboratory techniques?
2. Due to the lack of robust data on the prevalence of TSEs in small ruminants, the SSC is asked, as part of the follow-up to the SSC opinion of 18-19 October 2001 on the safety of small ruminant products, to propose a statistically valid sample design and size for a survey of TSEs in small ruminants. The survey should provide information on the current prevalence of TSEs in small ruminants, information for the assessment of the level of possible risk, if any, to consumers in a given country and information on the age-distribution of TSEs in small ruminants. Furthermore, the proposal should include the design and sample size for a survey of the TSE resistant genotypes in the sheep population.

A Working Group was created to prepare a scientific report to serve as a basis for replying the above questions. Its report is attached.

---

[1] The Geographical BSE-risk (GBR) is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, pre-clinically as well as clinically, at a given point in time, in a country. Where its presence is confirmed, the GBR gives an indication of the level of infection.

Key-words: TSE, BSE, surveillance, statistics, sampling, rapid TSE tests, fallen stock, emergency slaughter.

The Scientific Steering Committee provides the following answers to the various parts of the mandate. These answers do not cover aspects such as, for example, the definition of BSE status categories or threshold levels of BSE prevalence that would be acceptable in terms of public health. The choice of these levels lies with risk managers. To apply the outcome of the survey in terms of BSE status categorisation, criteria need to be developed and adopted to establish correspondence between BSE status categories and statistical prevalence, probabilities and confidence intervals.

A. Requirements for conclusive statistical surveys to be used as a basis for the classification of a country according to its BSE status or, as appropriate, for surveying of TSEs in small ruminants so as to obtain information on their current prevalence.

A.1. Sample design and size.

The valid interpretation of data from any TSE surveillance programmes (e.g., for risk assessment) depends on the sampling being effectively random for the whole target population.

The table hereafter indicates how to calculate the sample size as a function of probability levels and TSE prevalence. The table also indicates that it is not excluded that, for relatively small national herds or flocks or if a high precision is needed, more than one year will needed to reach the required sample size.

TSEs have long incubation periods (mean of 5 years for BSE in cattle) and the number of cases may be significantly different before or after major risk management measures (e.g., a feed ban). This should be considered when defining the target animal population and the temporal distribution of a survey.

Table : Sample sizes for TSE detection according to likely prevalence and probability level

Prevalence p_0	Probability of finding at least 1 TSE test positive 90%	95%	99%
For example:			
1/10,000,000	23,000,000	30,000,000	46,000,000
1/1,000,000	2,300,000	3,000,000	4,600,000
1/100,000	230,000	300,000	460,000
1/50,000	115,000	150,000	230,000
1/20,000	46,000	60,000	92,000
1/10,000	23,000	30,000	46,000
1/5,000	11,500	15,000	23,000
1/2,000	4,600	6,000	9,200
1/1,000	2,300	3,000	4,600

A.2. Target populations.

- For cattle, the minimal - and at least in theory sufficient - requirement, is the establishment of a statistically sound surveillance program for BSE in fallen cattle, sick slaughter and emergency slaughter animals (so-called risk stock) over the minimal age from which BSE has a reasonable chance to be detected if it is incubating. The reason is that, as an average for the whole EU, the BSE prevalence in this risk population is roughly 10 to 15 times higher than in healthy adult bovines offered for normal slaughter. This prevalence ratio may however vary for individual countries and according to the age from which animals are submitted to testing. (Details are given in the attached report). The variability between countries of the prevalence ratio is partly due to aspects such as the age limits used, the destruction schemes in place and the reliability of identifying/sampling risk stock. Greater experience of active BSE surveillance across countries is needed before a formal analysis along these lines could be proposed. Currently, for the EU, the median ratio of upper 95% confidence limit for BSE prevalence in risk versus healthy stock is approximately 10.

  The testing of risk animals is to be done initially for animals as from 24 months[2], but

[2] The probability to find BSE is probably nevertheless higher in older animals and the age limit could thus in certain cases be raised for example should for any reason the sample size be significantly smaller then recommended.

this threshold may need to be revised should it appear that the age distribution in fallen stock is different from clinically diagnosed BSE or from test positives among apparently healthy cattle offered for slaughter.

- **With regard to small ruminants,** the practicalities of a TSE rapid test surveillance may be different from cattle. Except if risk animals can reliably be traced and sampled, the animals would need to be sampled from those sent for slaughter, which implies much higher sample sizes than for cattle if risk population is sampled. In theory, there is no age cut-off[3], but initial surveillance should target the age-group in which TSE test positivity is most likely, namely adults (above 12 months)[4].

  Active surveillance will provide a prevalence rate among tested animals. For small ruminants, however, the unit of real interest for analysing TSE prevalence is currently the flock or farm. The set-up of a test programme should thus be such that positive test results can be linked to the farm or flock of origin. This should also be the case for animals sent for disposal, e.g., via rendering plants. In a second step, if test positive animals are found, and depending on the prevalence rate observed, a complementary surveillance design could be targeted at farms in order to quantify the percentage of affected ruminants per affected farm.

B. Sample size, taking into account possible temporal and geographical variation in challenge.

- Variations in BSE/TSE prevalence may occur between regions, birth cohorts, farm types, breeds, genotypes, etc. within the same country and over time, particularly in relation to the incubation period length. The sample size will depend on the choice of prevalence that should be detected in the target population and the confidence or reliability for decision-making. The target (cattle, sheep, goats) population can be a clearly defined age-restricted, national or regional or other sub-population

---

3 See the SSC Preliminary opinion of 6-7 September 2001 on Stunning methods.

4 The selected ages of the animals to be sampled may depend upon which tissue is being tested: if validated test are available that routinely can be applied to tissues such as tonsils, spleen or lymph nodes, animals below 12 months could be tested.

If information is needed about TSE prevalence in different subgroups of the target population (sub-grouping by age or region for instance) then separate sampling schemes would need to be set up, specifying the choice of prevalences to be detected and confidence levels separately for each subgroup. The populations of animals born before and after the full implementation of a feed-ban would constitute two separate, important sub-populations, to be considered separately for surveillance purposes.

- If a random sampling scheme was set up for the herd or flock of a given area, the results should be interpreted at the scale of that area. A sub-area-wise look within these results implies a smaller sample size per sub-area and therefore a decrease in the level of precision (confidence and/or prevalence).

C. The level of possible risk, if any, to consumers in a given country resulting from TSE in small ruminants.

(The section hereafter only addresses the prevalence of BSE in small ruminants, should it be present. The broader context of risks for consumers is addressed in the various SSC opinions on TSEs in small ruminants.)

With the currently available rapid tests (November 2001), BSE surveillance of adult animals has to proceed in two stages: rapid TSE testing to identify TSE positives, and a second form of testing [to be determined, but preferably shorter duration than transmission studies in mice[5]] used to discover if any TSE positives were in fact BSE positive. From the above table it can be derived that, to exclude a BSE prevalence in TSE rapid test positive adult sheep of 1 in 200 TSE test positives, a Member State would need to apply second-stage BSE testing to between 600 (95%) and 920 (99%) TSE rapid test positives without finding any TSE positive animal which is BSE positive. To exclude a BSE prevalence in TSE rapid test positive adult sheep of 1 in 2000 TSE test positives, a country would need to apply second-stage BSE testing to between 6000 (95%) and 9200 (99%) TSE rapid test positives.

5 The SSC is currently preparing a specific opinion on this subject.

D. Genotyping of small ruminants.

In its opinion of 18-19 October 2001 on BSE in small ruminants, the SSC recommended to associate a genotyping programme with the TSE rapid test surveillance in small ruminants. This permits to (a) adequately map scrapie susceptible sheep genotypes per country, and (b) confidently identify scrapie resistant sheep genotypes per country.

To rapidly and efficiently enhance knowledge about susceptible and resistant genotypes per country and to gradually conclude on the relation between genotype and TSE susceptibility, the SSC recommends that:

a) A random sub-sample of 500 from the first 100.000 adult animals which are subject to rapid TSE testing in a given country is genotyped and ideally flock-identified. The statistical rationale for 500 genotyped native animals per country is that there is then less than a 1% chance that a genotype with country frequency of 1% or more would fail to be represented.

b) Every rapid TSE test positive adult animal is genotyped together with sets of 6 suitably sampled controls per TSE positive case. (Countries which have not excluded that their TSE prevalence is 50 or more per 1 million adult sheep should continue rapid TSE surveillance until they have genotyped at least 100 TSE test positive adult sheep together with their associated controls per case.)

Such an associated programme would make available for high TSE prevalence countries a sample of 100 genotyped TSE test positive animals and 600 selected controls in addition to the random sample of 500 genotypings.

E. Collateral measures to be taken to ensure validity of the data in terms of, for example, collection and sampling and laboratory techniques.

The following collateral measures can be listed (non exhaustive):

E.1. Quality of implementation and measures against diversion.

If the target sub-population consists of risk animals (e.g., mostly in the case of cattle), then the testing of healthy stock in parallel with risk stock is recommended for at least the first year of active TSE surveillance in order to monitor the quality of implementation of

surveillance programmes. Thereafter active surveillance at slaughterhouses is needed to guard against diversion: the probability of being TSE rapid tested in that sample should be large enough to discourage the use of normal slaughter as a route for disposal of suspect animals.

If the target sub-population consists of animals sent for slaughter (which may be the case of small ruminants) then escape routes, such as channelling suspect animals for unmonitored disposal, should be controlled.

E.2. Quality assurance.

a) Practically oriented protocols ~~**instructions**~~ that ensure random sampling and population representative results should be scientifically sound, properly documented and preferably peer-reviewed.

b) Reporting format. A common reporting format for TSE cases is needed and future reporting should be done as soon as possible according to that format. Ideally, the surveillance database should differentiate clinical TSE from TSE test positive animals (normal slaughters, eradication, risk stock) and imported from native animals and record: month and year of birth, cause of death (normal slaughter, eradication, fallen stock, casualty, emergency slaughter, …), month and year of slaughter or death, age at slaughter or death, region of slaughter or death, TSE rapid test result and type of test used, and flock, farm and genotype (for ovines and, if appropriate, caprines).

In case of animals culled in the frame of a TSE eradication program, the data base should record whether the BSE-eradication was associated with a) clinical BSE or b) BSE test positive. It should also specify the nature of association as i) dam, ii) calf, iii) birth cohort and iv) farm cohort. As the definition of "eradication" animals may differ from country to country depending upon the culling strategy applied, definitions of what is covered by "BSE-eradication bovines" should accompany the above data base.

c) The whole survey system (including TSE rapid tests for use in surveillance programs, the laboratories which deploy them, the protocols, the records, etc.) should be subject to regular and formal quality assurance.

SCIENTIFIC REPORT ON REQUIREMENTS FOR STATISTICALLY AUTHORITATIVE TSE SURVEYS

TABLE OF CONTENTS:

6 Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999.

I. TERMS OF REFERENCE

In the light of the current TSE rapid testing possibilities, of the recommendations of the individual country geographical BSE risk (GBR) reports adopted by the SSC in July 2000 and the lack of exact data on the prevalence of TSEs in small ruminant populations, the SSC was invited to address the following questions:

- *What are the requirements for a conclusive statistical survey to be used as a basis for the classification of a country according to its BSE status?*
- *Which sub-populations should be tested and at what sample size? Is it possible to focus the sampling on certain sub-populations only? The sample size should be adapted to practical circumstances, such as possible temporal and geographical variation in challenge.*
- *Are there collateral measures that have to be taken to ensure validity of the data in terms of, for example, data collection and sampling and laboratory techniques?*
- *As part of the follow-up to the SSC opinion of 18-19 October 2001 on the safety of small ruminant products, the SSC is asked to propose a statistically valid sample design and size for a survey of TSEs in small ruminants. The survey should provide information on the current prevalence of TSEs in small ruminants, information for the assessment of the level of possible risk, if any, to consumers in a given country and information on the age-distribution of TSEs in small ruminants. Furthermore, the proposal should include the design and sample size for a survey of the TSE resistant genotypes in the sheep population.*

Hereafter follows the scientific report of a Working Group set up to prepare a scientific basis for replying to the above questions.

Key-words: BSE, surveillance, statistics, sampling, rapid TSE tests, fallen stock, emergency slaughter

II PREAMBLE

As requested in the mandate, the current report only addresses requirements for surveillance programs which improve the basis for future assessments of the geographical TSE risk, or help to verify the current TSE risk assessments. The report can therefore only be exploited in that context. It does not address the issue of testing of animals in the context of individual consumer protection.

III BACKGROUND

According to Article 5 of Regulation (EC) No 999/2001 Member States and third countries shall be classified into 5 categories according to their BSE status. The procedure starts with a risk assessment followed by the assessment of certain defined criteria. However, if the Commission finds that the information submitted is insufficient or unclear, it may, in accordance with the Regulatory Comitology procedure, determine that BSE status on the basis of a full risk analysis, which must include a conclusive statistical survey of the epidemiological situation in the country concerned. Such screening procedure may also be used by countries which wish to have the classification they carried out on that basis approved by the Commission.

Systematic rapid testing of cattle has been compulsory in the EU since January 2001. Until July 2001 the following groups of bovine animals over 30 months of age were subject to compulsory testing:

1) dead on farm or in transit: random sample of given size fixed by legislation.
2) Emergency slaughter 100%
3) Sick at normal slaughter 100%
4) Healthy animals for human consumption 100%, with derogations for Austria, Finland and Sweden.

Other animals could be tested on a voluntary basis.

Since July 2001, the following groups of bovine animals are subject to testing:

1) Healthy animals subject to normal slaughter for human consumption over 30 months of age –100% (Austria, Finland and Sweden –a random sample of 10,000 animals per year)
2) Emergency slaughtered animals and animals found sick at normal slaughter for human consumption over 24 months of age –100%
3) Animals killed under the OTMS scheme:
   -emergency slaughter and sick at ante mortem –100%
   -born between 1/8/1996 and 1/8/1997 –100%
   -a random sample of 50,000 animals per year of the other OTMS animals
4) Other animals over 24 months of age not slaughtered for human consumption (mainly animals dead on farm or in transport) –100% for a one year period, thereafter at random (sample of given size fixed by legislation).

The monitoring of the animals referred to in 2) and 4) will be reviewed after 6 months of testing.

The above monitoring program was laid down both for surveillance purposes and to protect the consumer. The permanent program (random sample in the animals referred to in (4)) is not intended to be used for the assessment of the BSE status of a country. However, the one-year 100% sampling of the animals referred to in 2) and 4) has been laid down as a minimum requirement for a statistical survey to confirm or overturn the GBR assessment.

IV. STATISTICALLY JUSTIFIED SAMPLE SIZES PER IDENTIFIED SUB-POPULATION

From a statistical point of view sample size calculations depend on the purpose of sampling, as follows:

IV.1. Sampling for detection of disease in a population:

'Detection of disease' means that at least one sample unit or animal tests TSE positive. Where the statistical problem is to test $H_0 : p \geq p_0$ (prevalence in population is equal or greater a critical prevalence p_0) against the alternative $H_1 : p < p_0$ (prevalence in population is less than a critical prevalence p_0) and the decision rule is to reject H_0 if 0/n animals are TSE positive, then:

depending on given values for:

- p_0 — agreed prevalence value that defines a population as positive, and
- α — probability of a type I error (a false rejection of H_O), i.e. the probability not to detect a positive population,

the minimum sample size n can be determined that allows for a statement like this: "With a probability of $(1 - \alpha)$ we can expect to sample at least one positive animal if the actual prevalence, in a sub-population, is greater or equal p_o." The next section provides more details on the calculation of sample sizes.

IV.2. Sampling for interval estimation of prevalence in population:

An estimate is preferably given by an interval that ensures a prescribed confidence $(1 - \alpha)$ that the limits calculated from the sample enclose the true prevalence. Depending on given values for:

- the confidence level $(1 - \alpha)$,
- the precision, i.e. the maximum length of the interval, required for the intended purpose, and
- some information on where the real prevalence is expected to be found.

  the minimum sample size n can be determined that allows for an estimate meeting these conditions.

Prescribing:

- plausible or critical prevalence,
- desired confidence in estimates for decision making,
- and precision of estimates,

needs careful consideration of the context.

Underpinning a) and b) are the following considerations about infinite theoretical population (see also Annex) and the distribution of the number, B, of TSE positive animals in a sample of size n.

The animal population considered in the current document is not the finite population

present in a given country during a given year. It is rather the (infinite[7]) super-population consisting of the whole of the animals that exist in reality or could be produced in the future, in a given region, under conditions that are similar to the current ones. This implies that animals born before and after major changes, such as a complete feed-ban, belong to different sub-populations and therefore should be sampled independently.

With the foregoing infinite population in mind, the number, B, of TSE test positive animals in a sample of size n follows a binomial distribution with parameters n and p, p being the proportion of positive animals in the population. It can be approximated by a Poison distribution with expectation n p.

IV.3. Sample size determination:

IV.3.1. Disease detection

The following calculations are based on the assumption that the prevalence of BSE in cattle can be described in terms of a binomial distribution (or Poisson approximation). In general, the sample size required to detect - with a given probability of at least $(1 - \alpha)$, for example a 95% probability - at least one positive animal if the true prevalence is p_0 or higher can be calculated as:

$$n \geq \frac{\log \alpha}{\log (1 - p_0)} \qquad [*]$$

for example: $n \geq \frac{\log 0.05}{\log (1 - p_0)}$

This calculation yields the sample sizes *n* listed in **Table 1**.

The above formula [*] can be inverted as follows. If a Member State has observed 0 TSE positives out of n sampled animals [that is: 0/n tested BSE positive] then the Member State can report that if BSE prevalence were higher than :

$$p_0 = 1 - \alpha^{\frac{1}{n}}$$

the chance of observing 0/n TSE positives would have been α% or less.

[7] In practice, within the context of the current report: larger than approx. 10,000 animals.

Table 1: Sample sizes for TSE detection according to likely prevalence and probability level

Prevalence p_0	Probability of finding at least 1 TSE test positive: 90%*	95%*	99%*
For example:			
1/10,000,000	23,000,000	30,000,000	46,000,000
1/1,000,000	2,300,000	3,000,000	4,600,000
1/100,000	230,000	300,000	460,000
1/50,000	115,000	150,000	230,000
1/20,000	46,000	60,000	92,000
1/10,000	23,000	30,000	46,000
1/5,000	11,500	15,000	23,000
1/2,000	4,600	6,000	9,200
1/1,000	2,300	3,000	4,600

*: at most a 10 %, 5% or 1 % chance that nil / n positives would have been observed

Examples:

- Suppose 30,000 animals have been sampled and none of them tested positive. If the true prevalence had been 1 case [or more] per 10,000 then there is less than a 5% chance that NIL positives would have been found (by inversion of [*]).

  A country such as Sweden which, between April and August 2001 had tested only just over 10,000 risk stock without finding any BSE positives, could only claim that if its true BSE prevalence had been 2.8 cases [or more] in 10,000 there was less than a 5% chance of observing NIL positives. Indeed, Table 5 gives the upper 95% confidence limit for Sweden's BSE prevalence in risk stock as 340 per 1 million.

- If BSE prevalence in adult sheep is greater than 1 in 100,000, then there is only a 1% chance that TSE testing of 460,000 adult sheep will fail to find any BSE by (subsequent) differential diagnostic testing.

- Equally, if the prevalence of BSE test positives in OTMS 5 year old bovines from UK's 1996/97 birth cohort is less than 1 in 100,000 then the expected number in 10,000 testees would be less than 0.1 and in that case the probability of observing 1 or more BSE positive testees in 10,000 [or 5000 or 3000] would be less than 0.10 [0.05 or 0.03]; and the probability of observing 2 or more BSE positives in 10,000 [or 5000 or 3000] testees would be less than 0.005 [or 0.001 or 0.0005].

- A final example concerns the monitoring separately of BSE prevalence in, say, 5 year old healthy bovines born before versus after the real feed ban of 1 August 1996 in UK. Suppose that before the real ban, BSE positivity in OTMS 5 year olds was 5 in 1000 and that the real feed ban had instituted at least a 50-fold reduction to 1 in 10,000. Then UK would have to test 46,000 5-year olds born after 1 August 1996 without finding any BSE positives in support of the assertion that there was less than

a 1% chance of observing NIL positives if the BSE prevalence in 5 year olds born after the real ban was 1 case [or more] in 10,000.

IV.3.2. Confidence intervals

More generally, since surveillance to date shows BSE prevalence in apparently healthy adult cattle to range from 10 to 100 per million adult bovines in most Member States, it is more appropriate to compute a 95% confidence interval for BSE prevalence in testees as approximately:

$$[(B-2\sqrt{B})/\text{number tested}] \text{ to } [(B+2\sqrt{B})/\text{number tested}]. \qquad [**]$$

For an approximate 99% confidence interval, the value of 2 is to be replaced by 2.58. When B [= number in sample which were BSE test positive] is 10 or more, the approximation [**] works well. When B is under 10, more exact Poisson methods should be used. **Table 2** hereafter provides the upper 95% and 99% confidence limits when B = 0, 1, . . . 9 .

A minimum sample size n can be determined so that there is 80% chance that the width of the above 95% confidence interval is less than a prescribed width. As a general guide, halving the width of a 95% confidence interval requires 4 times as many animals to be tested. This guide is fairly rough if B is under 10 because the 95% confidence interval is then generally asymmetric [see **Table 2**].

Table 2: 95% and 99% confidence limits for test positives when B = 0, 1, . 9.

B (Observed)	95% confidence limits		99% confidence limits
	Lower	Upper	Upper
0	0	3.7	5.3
1	0	5.6	7.4
2	0.2	7.2	9.3
3	0.6	8.8	11.0
4	1.1	10.2	12.6
5	1.6	11.7	14.2
6	2.2	13.1	15.7
7	2.8	14.4	17.1
8	3.5	15.8	18.6
9	4.1	17.1	20.0

Examples:

- If UK's OTMS testing of healthy 5 year olds born after the real feed ban found 3 BSE positives in 10,000, then Table 2 would suggest that a 95% confidence interval for the true BSE prevalence in such 5 year olds was from 0.6 to 8.8 per 10,000 or from 60 to 880 per 1 million 5-year olds. To halve the width of this confidence interval would require the testing of at least 40,000 5-year olds from the 1996-97 birth cohort. For example, if 8 BSE positives were found in 40,000 testees, then the 95% confidence interval for the true prevalence would be from 3.5 to 15.8 per 40,000 or

from 88 to 395 per 1 million.

- Other examples are given further on in this report.

V. DISCUSSION

V.1. MINIMAL REQUIREMENTS FOR INTENSIVE SURVEILLANCE PROGRAMS IMPROVING THE BASIS FOR FUTURE ASSESSMENTS OF THE GEOGRAPHICAL BSE RISK (GBR) IN CATTLE, OR HELPING TO VERIFY THE CURRENT RISK ASSESSMENTS.

The minimal - and at least in theory: sufficient -requirement for intensive surveillance programs which improve the basis for future assessments of the geographical BSE risk (GBR), or help to verify the current geographical BSE risk assessments, is the establishment of a statistically sound surveillance programme for BSE in fallen cattle sick slaughter and emergency slaughter animals over the minimal age as from which BSE has a reasonable chance to be detected if it is incubating.

The justification for the above is given in the sections hereafter.

V.1.1. The Scientific Report[8] on "Fallen Stock" (E.C., 1999)

In its Scientific Report[9] on "Fallen Stock" (E.C., 1999) the SSC stated:

"(...) A key-question to be addressed is whether the BSE prevalence and infectivity in fallen cattle is likely to be higher or lower than in the animals sent for slaughter and passing the ante- and post-mortem inspections.

One could argue that in many countries bovines are normally kept under conditions which imply a daily observation of the health status of the animals. A significant part (but certainly not all) animals that show neurological symptoms are likely to be reported on and killed following an active intervention of a veterinarian, especially if an adequate epidemio-surveillance system is in place. (…)It might therefore be considered unlikely that the numbers of animals that would die suddenly as a result of terminal BSE would be high. This would imply that the prevalence of BSE in fallen stock and dead animals entering the rendering chain is not higher than in the rest of the cattle population.-

However, it must be stated that, as a general principle, any disease has a higher probability to occur in fallen stock and dead animals than in apparently healthy animals sent for slaughter. Most animals are slaughtered before signs of illnesses may develop. This relates to diseases like slow virus infections or tuberculosis. No study has permitted an assessment of the epidemiology of TSE infection in animals found dead and nothing is known about the potential associations between a given TSE pathology and the consequences of its association with an infection with unconventional transmissible agents. (…)

The potential BSE infectivity risk associated with fallen stock and dead animals is therefore to be assumed to be higher than for animals sent for slaughter. (…)"

---

8 Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999.

9 Submitted to the Scientific Steering Committee at its meeting of 24-25 June 1999.

V.1.2. Outcome of the TSE rapid testing programs in bovines launched since 1999.

a. Data prior to 2001:

In France 30491 risk cattle have been tested in a pilot project carried out in Westren France between August 7 and December 22 2000 (C.Ducrot, pers. Comm, 2001) with one of the validated "rapid tests" for the presence of PrP-res in the brain. Animals were belonging to 3 categories: 1) fallen stock tested animals, 2) euthanized animals for medical reasons, and 3) emergency slaughtered animals for medical reasons. Among them, 49 were found positive by the rapid test and confirmed by one of the reference methods (western blot, neuropathology, immunohistochemistry). The global prevalence rate in the 30491 animals was 1.6 per thousand (95% confidence interval 1.2 - 2.1 per thousand) which is close to the **Swiss observations** reported in 1999 and 2000. Prevalence was 2.4 per thousand in emergency slaughtered animals, 1.0 per thousand for fallen stock animals and 3.0 per thousand for animals euthanised for medical reasons. Most of the cases were born in 1993, 1994 and 1995, respectively 3.3, 7.1 and 3.2 per thousand.

Some preliminary results from France, comparing data from the Mandatory Reporting System (32 cases) and the pilot program test on cattle at risk (49 cases) (from August 7 to December 22, 2000 in the same region) show the modal birth-cohort of the BSE clinical and rapid test cases (94-95, 5 years old), but the test cases were on average a bit older than the BSE clinical cases found with the MRS.

b. January- March 2001 data:

At the level of **the European Union**, a preliminary analysis of the January-March 2001 results of the EU rapid testing program showed that, in the previously mentioned risk sub-populations, BSE prevalence was as an average for the whole E.U., 10 times higher in cattle population at risk compared to cattle sent regularly to the slaughter house. (**Table 3**). (It should be noted that differences exist in the monitoring programmes run in different Member States: the purchase for destruction scheme was implemented differently and, in addition, some Member States have tested more animals than the minimum requirements)[10]. This ratio may vary for individual countries as is evident from subsequent surveillance data.

c. April- August 2001 data:

During April to August 2001 surveillance improved and the BSE prevalence rate ratio for risk:healthy stock was around 15 on average for the EU (ranging from 50 for France and Ireland to around 20 for Portugal, Spain and Germany but around 10 for Belgium, The Netherlands and Italy (**Tables 4 and 5**). In **Table 4**, it may be noticed that Member States' monthly number of healthy bovines tested per 1 million adult cattle shows less heterogeneity than in **Table 3** as surveillance has been implemented more fully. Exceptions, besides UK for which no data were available, are Portugal, which has an OTMS scheme similar to UK's, Spain, Italy and Greece and also

10 See for example Table 3 showing major differences in Member States' monthly number of healthy bovines tested per 1 million adult cattle and in monthly number of risk animals tested per 1 million adult cattle.

Finland and Sweden which took advantage of derogations. There is also greater regularity in the monthly numbers of risk stock tested per 1 million of adult cattle. Exceptionally low and high numbers were reported respectively from Belgium and Germany.

Table 3: Pilot BSE rapid testing in EU in January to March 2001

country	# bovines > 2 years of age [in millions]	Monthly # slaughtered & BSE tested per 1m.	Monthly # casualty & BSE tested per 1m.
United Kingdom (UK)	5.30	*	*
Ireland (IRL)	3.43	4,900 [Feb.]	250 [Feb.]
France (F)	11.04	14,200	510 [Jan.]
Portugal (PT)	0.79	*	*
Spain (ES)	3.38	4,900	680 [Feb.]
Germany (DE)	6.57	16,100	2900
Belgium (B)	1.49	15,600	290
Netherlands (NL)	1.83	16,900	1780
Italy (I)	3.40	2,600 [Jan.]	480 [Jan.]
Denmark (DK)	0.94	22,300	1220
Austria (AU)	1.03	14,700	790
Luxembourg (Lux)	0.10	16,400 [Feb; Mar]	2330 [Feb; Mar]
Greece (GR)	0.34	2,500 [Feb; Mar]	140 [Feb; Mar]
Finland (Fin)	0.43	2960	*
Sweden (SV)	0.73	2660	*
BSE testing per 1m bovines > 2 years of age and BSE positivity in priority groups			
TOTAL: Jan. {Be, De, Ge, Sp, Fr, It, Ne, Au.} BSE positivity in eradication stock = 2/3234	30.40	13,200 BSE positivity per 100,000 = 4.7 [19/401262]	910 BSE positivity per 100,000 = 58 [16/27766]
TOTAL: Feb. {Be, De, Ge, El, Sp, Ir, Lu, Ne, Au.} BSE positivity in eradication stock = 1/4526	19.12	11,400 BSE positivity per 100,000 = 8.2 [18/218446]	1430 BSE positivity per 100,000 = 81 [22/27318]
TOTAL: Mar. {Be, De, El, Fr, Lu, Ne, Au.} BSE positivity in eradication stock = 0/ 919	15.74	15,300 BSE positivity per 100,000 = 4.7 [11/240863]	820 BSE positivity per 100,000 = 31 [4/12911]

*: data not available or not yet exploitable

Based on nil BSE test positives out of 82,082 healthy bovines tested in Austria during April to August 2001, the upper 95% confidence limit for Austria's BSE prevalence in healthy bovines is 45 per 1 million; and is 29 per 1 million based on nil positives out of 127,458 healthy bovines tested from January to August 2001.

Based on nil BSE positives out of 3,572 risk stock tested in Austria during April to August 2001, the upper 95% confidence limit for Austria's BSE prevalence in risk stock is 1036 per 1 million. If an EU-common BSE prevalence rate ratio of 15 [currently][11] were applied, the implied upper 95% limit for healthy stock would be 1036/15 = 69 per 1 million healthy stock. Clearly, in Austria's case, with only 690 risk bovines tested per month per 1 million adult bovines, greater precision has been achieved by the direct testing of healthy stock.

In the case of Denmark, where 1/89,821 healthy stock tested BSE positive during April to August 2001, the upper 95% confience limit for Denmark's BSE prevalence in healthy bovines is 62 per 1 million; and is 37 per 1 million based on 1/152,840 healthy stock having tested BSE positive during January to August 2001. Based on nil BSE positives out of 7,308 risk stock tested in Denmark during April to August 2001, the upper 95% confidence limit for Denmark's BSE prevalence in risk stock is 3.7/0.007308 = 506 per 1 million.

For Germany, where 13/980,352 healthy stock tested BSE positive during April to August 2001, the upper 95% confidence limit for BSE prevalence in healthy stock approximates $\{13 + 2 * \sqrt{(13)}\}/0.980352 = 21$ per 1 million; and is $\{35 + 2 * \sqrt{(35)}\}/0.123421 = 379$ per 1 million in risk stock.

It may thus be concluded that the prevalence of BSE in fallen stock and other risk sub-populations is higher than in healthy looking animals offered for normal slaughter. Therefore the results of a statistically sound sampling scheme applied to the sub-populations with the highest prevalence will a priori be a reliable "worst case" indicator for the prevalence of BSE in other sub-populations, provided of course there is no temporal change in which sub-population has the highest incidence.

d. <u>Rapid test results in eradication data</u>.

The number of BSE eradication bovines per country will depend on the number of its clinical BSE cases, its BSE positivity in fallen or emergency slaughter stock, and how its eradication protocol has been specified. Within and between country comparisons of the age-distribution for BSE positives identified among risk versus BSE-eradication stock versus clinical BSE will be important. It is unclear how many BSE-eradication stock are to be expected [30 or 100 or 150 or more?] per clinical BSE case, or per rapid test BSE positive. For EU, assuming 4000 BSE clinical or test positive cases and 100 BSE-eradication stock per positive, then the BSE-eradication program could account for 400,000 rapid tests per annum EU-wide, and around 150 further positive tests[12]. Further work is needed on the documentation and practice of rapid BSE testing in eradication stock.

---

[11] Warning: The given EU-common rate ratio of 15 is a rough guide only because part of the tested animals was younger than 30 months, in certain countries even below 24 months

[12] Uncertain estimate because definition of eradication stock and completeness of their BSE rapid testing and reporting may differ between Member States.

A comparative analysis of the rapid BSE test results in eradication stock has so far not been possible because eradication measures vary according to the country and the interpretation of the test results in this sub-population depends upon the eradication scheme applied. Records should therefore mention the number of eradication animals per BSE clinical or test positive case so that BSE positivity rates between countries can be prepared**.**

Table 4: Performance per 1 million adult cattle per month (based on April-August 2001, (active surveillance)) (anomalies in bold)

Country	Adult cattle (m)	#healthy tested [per 1 million per month] [3]		#risk tested [per 1 million per month]		Ratio [per 1 million]
UK	5.30		(14,000)	9,844	[930] [1]	(15)
IRL	3.43	123,809	[18,000] [1]	5,707	[830] [1]	22
FR	11.04	929,351	[16,800]	24,383	[1100] [1]	15
PT	0.79	12,158	**[3,100]**	2,874	[730]	4
ES	3.38	116,619	[6,900]	16,334	[970]	7
DE	6.57	980,352	[29,800]	123,421	[3760]	8
B	1.49	137,068	[18,400]	**2,444**	**[330]**	**56**
NL	1.83	146,502	[16,000]	11,410	[1250]	13
I	3.40	133,943	[7,900]	28,822	[1700]	5
DK	0.94	89,821	[19,100]	7,308	[1550]	12
AU	1.03	82,082	[15,900]	3,572	[690]	23
Lux	0.10	9,543	[19,100]	394	[790]	24
GR	0.34	6,921	**[4,100]**	871	[510]	8
Fin [2]	0.43	3,002	[1400]	9,313	[4,330]	0.3[2]
SV [2]	0.73	1,138	[300]	10,877	[2,980]	0.1[2]

1 If based only on July & August 2001.

2 In the market measures in place January to June, which made testing in animals over 30 months for human consumption compulsory, Finland, Sweden and Austria could derogate and were in fact not required to test healthy animals. Finland and Sweden made use of the derogation. In the rules now in place in the framework of the TSE Regulation, Finland, Sweden and Austria may decide not to test all healthy cattle slaughtered for human consumption, but must instead test a random sample of 10,000 animals per year. Finland and Sweden are making use of this possibility.

3 Most countries test animals above 30 months, some countries test animals as from 24 months of age or even below 24 months. Such countries are expected to have a lower rate per million because the likelihood to detect pre-clinical BSE is very low in 24-29 month old bovines, including with current tests.

Table 5: BSE test positives & positivity rate (active surveillance) (anomalies in bold)

Country	Adult catlle (m)	#healthy tested[4] [#positive] & rate per 1 million (upper 95% cl)			#risk tested [#positive] & rate per 1 million (upper 95% cl)			Rate ratio[3]
UK	5.30				9,844	[39][1]	4000 (5231)	
IRL	3.43	123,809	[10][1]	81 (132)	5,707	[24][1]	4210 (5922)	50
FR	11.04	929,351	[24]	26 (36)	24,383	[29][1]	1190 (1631)	48
PT	0.79	12,158	[2]	165 (592)	2,874	[8]	2780 (5498)	17
ES	3.38	116,619	[5]	43 (100)	16,334	[15]	920 (1393)	21
DE	6.57	980,352	[13]	13 (21)	123,421	[35]	280 (379)	22
B	1.49	137,068	[10]	73 (119)	**2,444**	[1]	410 (2291)	6
NL	1.83	146,502	[2]	14 (49)	11,410	[1]	90 (491)	6
I	3.40	133,943	[10]	75 (122)	28,822	[6]	210 (455)	3
DK	0.94	89,821	[1]	11 (62)	7,308	[0]	(506)	
AU	1.03	82,082	[0]	(45)	3,572	[0]	(1036)	
Lux	0.10	9,543	[0]	(388)	394	[0]	(9391)[2]	
GR	0.34	6,921	[0]	(535)	871	[0]	(4248)[2]	
Fin	0.43	3,002	[0][2]	(1233)	9,313	[0]	(397)	
SV	0.73	409	[0][2]	(9046)	10,877	[0]	(340)	
Total without UK[4],FN[5] &SV[5]		**1.84m**	[53]	**28.8**	**0.212m**	[90]	**424**	15

1 If based only on July & August 2001. (For UK it is assumed that two thirds of the pending cases will test BSE positive. (UK's update at 5 November 2001 shows that 111/21928 risk stock tested in GB during July to September 2001 were BSE positive, that is: 5062 per million [upper 95% confidence limit: 6023] so the estimate for UK is conservative.)

2 Low numbers tested

3 The given rate ratios provide a rough guide only because part of the tested animals may have been younger than 30 months, in certain countries even below 24 months.

4 Most countries test animals above 30 months, some countries test animals as from 24 months of age or even below 24 months Such countries are expected to have a lower rate per million because the likelihood to detect BSE, with the currently available rapid tests, is very low.

5. Excluded because the surveillance periods for healthy and risk stock differ for these countries.

e. Note on BSE In the UK.

Based on nearly 500, mainly clinical, BSE cases in EU outwith UK in 2000, *ceteris paribus*, universal TSE rapid testing of risk stock and in apparently healthy bovines over 30 months offered for slaughter could be expected effectively to treble that total to 1500 in 2001. To UK's nearly 1450 clinical BSE cases in 2000 need to be added an estimated 2700 BSE test positives in OTMS [around 10,000 OTMS 5+ year old bovines were tested out of roughly 675,0000, 0.4% of them BSE test positive] and, by analogy with rest of EU, around 1400 BSE positives in risk stock [which were not tested in UK in 2000]. Assuming that UK's epidemic halved in 2001 [as per predictions [13]] compared to 2000, clinical BSE cases plus universal TSE rapid testing [had it been operational throughout 2001] of risk stock and in apparently healthy bovines offered for OTMS slaughter could have accounted for over 2500 BSE test positives. (See however also **section f** hereafter on coverage-adjusted reporting).

f. Coverage adjustment of the number of BSE cases.

Comparisons between Member States, or between reporting years per Member State, should be based on the Member State's coverage-adjusted BSE cases.

EU-wide rapid BSE testing in 2001 of healthy cattle, in risk stock and in eradication stock has been implemented differentially in Member States. At present, reporting of BSE clincal + BSE active surveillance cases is combined but, since active surveillance has been applied differentially across a country, a reporting supplement is required per country which indicates what proportion of a surveillance category its BSE active surveillance cases emanate from. This is illustrated by the two examples hereafter, the first taken from the UK OTMS scheme (**Table 6a** and the more general **Table 6b**). **Table 7** illustrates the reporting format which the Working Group recommends.

Table 6a : Coverage of BSE surveillance (Example: UK in 2000).

Member State : UK		Year of confirmation/BSE rapid test : 2000		
Surveillance category	Coverage	Population size	BSE cases	
			observed	adjusted
clinical	100%	*	1,400	1,400
active: OTMS, 5+ yrs	10,000	675,000	40	2,700
active: OTMS, other	0%	225,000	?	?
active: risk stock	0%	150,000	?	?
active: eradication	0%	?	?	?
Total :			1,440	4,100 or 5,500 **

*: not applicable

13 See the SSC opinion of 8 December 2000 on Monitoring Some Important aspects of the evolution of the Epidemic of BSE in Great-Britain (Update providing an epidemiological commentary on BSE projections for Great Britain (GB) and on surveillance, as well as on the occurrence of "Born After the Real Ban - BARB" cases)

** conservative assumption: by adding at least as many BSE test positives in risk stock as there are in clinical cases.

Table 6b : Coverage of BSE surveillance (hypothetical example)

Member State : yy		Year of confirmation/BSE rapid test : xxxx		
Surveillance category	Coverage	Population size	BSE cases	
			observed	adjusted
clinical	100%	*	60	60
active: healthy >30 months	100%	2,400,000	60	60
active: risk stock	50%	150,000	45	90
active: eradication	100%	?	?	?
Total :			165	210

*: not applicable

Table 7 : Reporting format for BSE clinical + BSE active surveillance cases.

Member State : …….		Year of confirmation/BSE rapid test : …….		
Surveillance category	Coverage	Population size	BSE cases	
			estimated	adjusted
clinical				
active: slaughterhouse > XX months				
active: risk stock				
active: eradication				
active: Other				
Total :				

Interval estimates can be used to qualify the above central estimates. For example, in the UK OTMS survey of 2000, 10.000 animals above 5 years were tested out of a total population size of 675,000 animals. 40 were found positive for BSE. The 95% confidence interval for number of BSE rapid test positive cases per 10,000 in OTMS, 5+ years is from 40 - 2 √(40) to 40 + 2 √(40), that is: from 27.3 to 52.6. Hence, 95% confidence interval for coverage adjusted BSE cases in this surveillance category is from 67.5 * 27.3 = 1840 to 67.5 * 52.6 = 3550 (rounded to nearest 10 BSE cases).

V.1.3. THE AGE OF THE SURVEYED/SAMPLED BOVINES.

The age at which clinical BSE is detected in cattle ranges between 20 months as lowest age recorded so far and over 19 years. The modal age at detection is 4 - 6 years. The proportion of BSE cases in UK cattle aged 24 months or less at onset was less than 0.006% (or 10 animals out of approx. 177,500 cases, all of them observed before 1997),

0.05% or 81 cases for animals of or under 30 months of age and 0.17% or 307 cases for animals of or under 35 months of age. For other countries much less data are available, as the BSE cases are so far very much more limited.

One could therefore argue that the testing of animals could be limited to animals above the age from which the likelihood to find a case, if present, is not negligible. In practice this age limit would be approx. 30 months for apparently healthy bovines offered for slaughter. However, the Working Group considers that it has not [yet] been [fully] verified:

a. that the age distribution of BSE cases outside the UK is similar to the UK. Although there are currently no indications to the contrary, it is indeed not excluded that the two age distributions are different for example because (part of) the BSE incidence outside the UK may be second or third generation cases or because the infectious doses to which the animals have been exposed were different inside and outside the UK.

b. that the age distribution of BSE in the sub-populations of risk animals such as fallen stock follows the same pattern as in the sub-population of living animals.

The Working Group therefore recommends that the sampling of animals from the sub-populations of risk animals (fallen stock, emergency slaughter, sick slaughter) is, in a first phase, done for all animals above 24 months, until sufficient and reliable data are available to determine the age distribution of the prevalent BSE test positives in these populations. The threshold is set at 24 months because the diagnostic tests available so far are capable to detect BSE agents only if the animal tested is in its final stage of incubation and because the number of cases in healthy animals below 30 months is very low. 24 months should in principle take into account a possibly higher representation of younger animals with BSE in fallen stock. It may need to be revised should it appear that the age distribution in fallen stock is different from healthy animals. Once this information is available, the application of an age-weighted sampling scheme or, if needed, sampling that allows for age-stratification could possibly be considered.

V.2. SURVEILLANCE IN SMALL RUMINANTS

V.2.1. TSE surveillance

- Scrapie in sheep is under-reported. Moreover, when clinical scrapie has been followed up by veterinary surveillance of the host flock or post-mortem testing, additional clinical or sub-clinical cases have been discovered, for example in Norway and in Belgium. (Ulvund, pers.comm., 2001 ; Vanopdenbosch, pers.comm., 2001). in sheep with non-resistant genotypes.

  Preliminary estimates from E.U. Member States suggest that if correction is made for under-reporting and the conservative assumption is made that there is at least one additional rapid TSE test positive adult sheep per scrapie case, then TSE prevalence in adult sheep could range from 20 to 500 TSE positives per 1 million adult sheep according to Member State.

  By analogy with cattle, TSE prevalence may be substantially higher in risk stock than in similarly-aged sheep which are being slaughtered for human consumption. Because

of their lower value, sheep are seldom sent for emergency slaughter, and may be killed on farm or die where they roam or sent directly to a rendering plant or disposal site. Thus surveillance of risk sheep, although feasible [and being undertaken in certain countries, for example in Switzerland], is unlikely to be comprehensive. The target group of risk animals in small ruminants is therefore not comparable to the corresponding target group in cattle.

Therefore, except if risk animals can reliably be traced and sampled, the animals would need to be particularly sampled from those sent for slaughter, which implies much higher sampling sizes.

- TSE rapid test surveillance in sheep and goats should in principle cover animals of all ages[14]. However, initial surveillance should target the age-group in which TSE test positivity is most likely, probably adults but this may depend upon which tissue is being tested: if validated test are available that routinely can be applied to tissues such as tonsils, spleen or lymph nodes, animals below 12 monts could be tested.
- The Working Group therefore recommends active rapid TSE test surveillance of adult sheep at slaughterhouses as the first step in improving scrapie surveillance in Member States. It is important that this surveillance targets primarily native sheep.

  An additional surveillance scheme for imported sheep may need to be considered by majorly importing member states. But in any case, escape routes for the discovery of a TSE in small ruminants, such as channelling suspect animals to the disposal chain, should be controlled.

  Second and third stages of active TSE surveillance may be envisaged, as follows:

  - surveillance based on rapid TSE testing in the spleen of sheep under 12 months which have been sent for slaughter. [Development of suitable tests is nearing completion and surveillance on this basis could proceed after appropriate validation and quality assurance has been undertaken].
  - surveillance based on flocks, because scrapie eradication policies are flock-based, and making use of genotyping and, potentially, tonsil-based TSE testing of live sheep to limit within-flock culling.

V.2.2. BSE surveillance

The surveillance goal is to determine if there is any BSE in flocks (as a fraction of overall TSE prevalence). With the currently available rapid tests (November 2001), BSE surveillance of adult animals has to proceed in two stages: rapid TSE testing to identify TSE positives, and a second form of testing [to be determined, but preferably shorter duration than transmission studies in mice[15]] used to discover if any TSE positives were in fact BSE positive.

From the example hereafter it may be concluded that to exclude a BSE prevalence in TSE

---

14 See the SSC Preliminary opinion of 6-7 September 2001 on Stunning methods.

15 The SSC is currently preparing a specific opinion on this subject.

positive adult animal of 1 in 200 TSE test positives, a country would need to apply second-stage testing to between 600 and 920 TSE rapid test positives without finding any TSE positive animal which is BSE positive.

Example: the interpretation of the strain typing results in the UK.

In UK, there may be up to 5000 clinical scrapie cases in 20 million adult sheep, and therefore probably at least 10,000 TSE positives per 20 million adult sheep, or up to 500 per 1 million adult sheep sent for slaughter.

Transmission studies in mice have ruled out BSE in 156 clinical TSEs in sheep [nil /156; upper 95% confidence limit for BSE prevalence in clinical TSEs in sheep = 3.7/156 = 2.4%]. Other testing has reportedly ruled out BSE in over 800 clinical TSEs in sheep [nil/800; upper 95% confidence limit for BSE prevalence in clinical TSEs in sheep = 3.7/800 = 0.5%].

If 2.5% [or 0.5%] of TSE positives in adult sheep in 2001 were BSE then UK's BSE prevalence in adult sheep might be up to 12.5 [or 2.5] per 1 million adult sheep.

To exclude a BSE prevalence of 10 per 1 million adult sheep at slaughterhouses [EU's threshold of concern for healthy cattle over 30 months], UK would have to test nearly 500,000 NATIVE adult sheep without finding any SHEEP which was BSE positive.

By the same token, and assuming TSE prevalence of 500 per 1 million UK adult sheep, to exclude a BSE prevalence of 1 in 400,000 adult sheep at slaughterhouses, over 1.8 million native adult UK sheep would need to be tested without finding any animal which was BSE positive.

V.2.3 Note on testing and genotyping (surveillance)

Rapid TSE surveillance of adult sheep might need to be more comprehensive: (a) to adequately map scrapie susceptible sheep genotypes per Member State, and b) to confidently identify scrapie resistant sheep genotypes per MS. Rapidly and efficiently to enhance knowledge about susceptible and resistant sheep genotypes per Member State, the Working Group further recommends that:

a) A random 0.5% sub-sample of the first 100,000 adult sheep which are subject to rapid TSE testing is genotyped [that is: randomly-selected 500 adult sheep per member state], and ideally flock-identified. The statistical rationale for 500 genotyped native adult sheep per Member State is that there is then less than a 1% chance that a genotype with MS-frequency of 1% or more would fail to be represented.

and:

b) Every rapid TSE test positive adult sheep is genotyped together with two sets of 3 suitably-sampled controls per TSE positive case:

- Control set i) = 3 [randomly sampled] TSE test negative adult sheep from the same slaughterhouse as the TSE test positive case, and
- Control set ii) = 3 [randomly sampled] adult sheep from the same flock as the TSE positive case.

Control set i) can be achieved from the outset of active TSE test surveillance because it only requires that the TSE rapid test laboratory knows from which slaughterhouse the ovine test samples have come. Control set ii) requires, in addition, a flock identifier per sample tested. Since this information is recommended to be collected anyway in respect of TSE test positive adult sheep, the surveillance system should ideally be set up so that a flock identifier is linked to every TSE rapid test sample. This may require some time for its organisation within Member States. Ensuring that a flock-identifier is linked to each specimen in the above 1% genotype sub-sample is likely to be logistically feasible from the outset.

Table 1 provides the numbers of adult sheep brains [over 12 months] to be rapid TSE tested at slaughterhouses without finding any which are TSE positive for a Member State to conclude that, IF its TSE prevalence in adult sheep were greater than p_o, then there is at most a 10%, 5% or 1% chance that *nil* positives would have been observed.

Interval estimation of TSE prevalence rates with adequate precision, rather than scrapie detection, is likely to be the surveillance goal in most EU Member States, (in stead of detecting absence / presence of TSE). It is, however, precisely because Member States with lower a priori-expected TSE prevalences in adult sheep [50 or less per 1 million adult sheep] may find few [if any] TSE rapid test positives in 100,000 tested adult sheep that the Working Party recommends that - in addition to the above genotyping of cases and their associated control sets i) and ii) - genotyping should also be investigated [and flock identified] for a random 0.5% sub-sample of the first 100,000 native adult sheep at slaughterhouses which are subject to rapid TSE testing. This ensures that, as an output from its rapid test surveillance programme, every Member State would have genotyping, and flock identification, available on at least 500 native adult sheep sent for slaughter.

Example**:**

UK has an estimated 20 million adult sheep with perhaps 4 million slaughtered per annum. Rapid TSE surveillance of 250,000 adult sheep could thus be achieved in less than 2 months by 100% surveillance at all slaughterhouses. If TSE positives in adult sheep were as high as 1/2,000 in UK, then TSE surveillance of 250,000 adult sheep would be expected to yield 125 TSE test positives to be genotyped, each linked to 3 slaughterhouse (control set i) + 3 within-flock (control set ii) adult sheep, so that genotyping would need to be performed on 125 case + 750 control sheep. If UK's TSE test positives in adult sheep were only 1/5000, then rapid TSE testing of 250,000 adult sheep would be expected to yield only 50 cases for genotyping, which may be too few for robust identification of susceptible genotypes.

V.2.4. Summary:

Rapid TSE surveillance in a minimum of 100,000 adult animals for a given Member State would indicate whether or not the TSE prevalence is above or below 50 per million animals **(see Table 1**) and for this prevalence there would be only a 1 % chance that nil / n positives would have been observed.

Genotyping of a 0.5% random sub-sample of 100,000 animals, per Member State, would

make available for each Member State an initial sample of 500 genotyped animals .

In addition, the Working Group recommends that Member States which have not excluded that their TSE prevalence is 50 or more per 1 million adult sheep should continue rapid TSE surveillance until they have genotyped at least 100 TSE test positive adult sheep together with their associated controls per case (3 slaughter-house matched adult sheep, 3 flock-matched adult sheep). This would make available for such a country a sample of 100 genotyped test positive animals and 600 controls, in addition to the above minimal sample of 500 genotypings, which would permit reliably to conclude on the relation between genotype and TSE susceptibility.

V.2.5. Some practical aspects:

- A 99% reassurance that BSE prevalence in adult sheep is less than 1 in 100,000 would require TSE testing of at least 460,0000 adult sheep without finding any BSE by (subsequent) differential diagnostic testing or strain-typing.
- Currently, the unit of interest controlling or eradicating TSE prevalence in small ruminants is the farm. The goal is to estimate the percentage of farms affected with TSE. The set-up of a rapid TSE test programme should thus be such that positive test results can be linked to the farm or flock of origin. This should also the case for animals sent for disposal, e.g., via rendering plants.

  Active surveillance is targeted at the animal level and will provide a prevalence rate among tested animals. In a second step, if test positive animals are found, and depending on the prevalence rate observed, a complementary surveillance design could be targeted at the farm in order to quantify the percentage of farms affected with TSE in a given country.

V.3. OTHER SUB-POPULATIONS TO BE TESTED

The Working Group suggests that due consideration should be given to test also (a) the 1997/98 birth cohort in the UK's OTMS and (b) a random sample of other bovines aged over **30** months[16] which are slaughtered for human consumption, or in UK's Over Thirty Month Scheme [OTMS].

The reasons why a sample of these sub-populations need to be tested are specified in the SSC opinion of 30.11.01 on the *Six BARB cases in the UK since 1 August 1996*.

V.4. COLLATERAL MEASURES THAT HAVE TO BE TAKEN TO ENSURE VALIDITY OF THE DATA IN TERMS OF, FOR EXAMPLE, COLLECTION AND SAMPLING AND LABORATORY TECHNIQUES.

a) Technical issues. The proposed Binomial distribution and calculated sample sizes

[16] Several opinions of the SSC indirectly address the age aspect as from which the *current* rapid BSE tests can be usefully applied for surveillance purposes. Given the facts that (1) the current test are *post-mortem* tests that are capable to detect BSE agent only in the final stages of incubation and that (2) the incidence of BSE in living animals below 30 months is very low, the usefulness of applying the currently available test on animals younger the 30 months is very limited if not negligible.

suppose a clearly defined target population or sub-population in terms of characteristics such as age distribution, region, period over which BSE prevalence is being estimated, feeding practices (e.g., dairy versus beef cattle), sex, breed, exploitation types, etc. From a statistical point of view it should therefore be stressed that the valid interpretation of sampling results (of decision making and estimation, as well as calculation of sample sizes) depends on the sampling being effectively random or on census being complete. Therefore the sampling protocols should be scientifically sound, duly documented and peer-reviewed and practically oriented instructions have to be elaborated that ensure random sampling and population representative results

b) Diversion

- **<u>cattle</u>**

  However, in spite of the fact that a correct sampling of risk poplualtions would be sufficient, the Working Group nevertheless considers that it would be unwise for there to be no TSE rapid testing at slaughterhouses when there is universal testing of casualty animals because that might risk anticipatory diversion of suspicious stock to slaughterhouses. In the above quoted report of 1999, the TSE/BSE *ad hoc* Group indeed further expressed *"its concerns about a report by Hart et al (1997), indicating that the number of reported fallen stock in the UK may have dropped by almost 50% for cattle and 80% for sheep, following the introduction of the ruminant feed ban and the reduced value of a fallen animal sold to a knacker. A not-confirmed hypothesis is that part of these animals are being buried on the farm, put in the slurry pit to rot, left to be picked clean (sheep) or, while still alive, declared as casualty. (In the United Kingdom: 2% of the cattle population is estimated to end up as fallen stock and casualty rates are also estimated at 2%. For sheep, the figures are approximately 1% and 3-5%.). This may indicate that under certain circumstances, the number of fallen stock that enter a rendering system – hence the potential infective load of a rendered batch - may drop in favour of other ways of disposal."*

  An additional sample of bovines over 30 months at slaughterhouses is thus needed to guard against diversion. The probability of being tested in the latter sample should be large enough (around one third) to discourage the use of normal slaughter as a route for disposal of suspect animals. This should be a random or - less acceptably -systematic[17], sample of bovines over 30 months.

- **<u>small ruminants</u>**

  In the case of small ruminants, escape routes (e.g., for disposal of suspect animals or cadavers) are not always controlled, and farmers with sporadic forms of scrapie can easily pass through the surveillance.

  In affected farms, it can be postulated that the percentage of sheep clinically affected per year varies from about 1% to several percents (10%?). The percentage

17 However, the systematic's sampling's very predictability [e.g. every 5th bovine] means that diversion can too easily occur [unless, every hour, say, a new random start for systematic one fifth sampling is made].

of adult sheep culled each year is about 15 to 20%, and the percentage of adult sheep that die each year is about 2 to 6%, but this may vary between countries and regions.

Considering *for example* 2% infected farms with 1% clinically affected sheep per year in infected farms, 0.2 per thousand adult alive sheep would be affected each year. If all the clinically affected sheep die and are sent to the rendering plant, and if 6% of adult sheep die every year and are sent to the rendering plant, up to 0.3% adult sheep could be found positive at the rendering plant.

In the context of human health and disease control, a surveillance program in small ruminants will thus require complementary aspects to control escape routes. This includes identification of the animals, control of the flock population and if the programme is mainly carried out on live (healthy slaughtered) animals, testing also a sample of dead and culled (risk?) animals that is sufficiently large to discourage this route of escape.

In many countries significant numbers of imported animals are slaughtered. Their origin should be adequately recorded and the results of the TSE rapid test surveillance programme should be reported separately for native animals.

c) Other collateral measures related to small ruminants.

For other collateral measures related to small ruminants: see Section V2.

d) BARB-control study. Any BSE positive risk stock, any BSE positive eradication stock and any BSE positive slaughterhouse stock born after the date of installation of a total feed ban or later should be followed up in accordance with SSC recommendations for an EU-wide BARB-controls study.

All BSE positive risk, eradication and apparently healthy stock should be followed up in accordance with the national protocol for identifying and culling at-risk bovines associated with the BSE positive case such as its dam, calves born up to 2 years before, and after, clinical onset in the dam or birth and farm cohort.

e) A common monthly reporting format for BSE rapid test program is needed. Ideally, the surveillance database should record: month and year of birth, cause of death (normal slaughter, eradication, fallen stock, casualty, emergency slaughter, …), month and year of slaughter or death, age at slaughter or death, region of slaughter or death, TSE rapid test result and type of test used.

In case of animals culled in the frame of a BSE eradication program, the data base should record whether the BSE-eradication was associated with a) clinical BSE or b) BSE test positive. It should also specify the nature of association as i) dam, ii) calf, iii) birth and farm cohort. Finally, records should mention the number of eradication animals per BSE clinical or test positive case so that BSE positivity rates between countries can be prepared**.** As the definition of "eradication" animals may differ from country to country depending upon the culling strategy applied, the definitions of what is covered by "BSE-eradication bovines" should accompany the above data base.

Notes:

- The availability of a common analysis and reporting software for application to common-format core databases would be desirable and should be made available to member states for local use as well as central analysis of the pooled core data.
- Retrospectively, age in months should be ascertained for the BSE test positives in the EU-wide testing program that started in January 2001 and future BSE test positives should be reported as soon as possible according to the above suggested lay-out**.**
- Of particular interest will be comparison, between the various EU Member States of the numbers of clinical BSE cases, BSE positives in risk stock, BSE positives in BSE-eradication stock, and BSE positives in slaughterhouses testing programs. Also of interest will be comparison of age distribution for the above four groups of BSE clinical/test positive cases.

f) Coverage-adjusted BSE comparisons between countries. Because active BSE surveillance has been introduced at different times and with different coverage per Member State or country (including because of derogations), robust international comparison requires that all countries should report annually their total of clinical + coverage-adjusted estimated total of rapid test positives.

g) Quality control. The TSE rapid tests for use in BSE surveillance program, and the laboratories which deploy them, should be subject to regular quality assurance.

V.5. NOTES AND PRACTICAL ASPECTS

a) **Ratio of BSE prevalence rate in risk stock / BSE prevalence rate in healthy looking stock.** The category of bovines aged over 30 months [24 months in certain countries or even below on a voluntary basis] slaughtered for human consumption, or in UK's Over Thirty Month Scheme [OTMS] represents roughly 10 times more stock per month than are accounted for as risk bovines. A preliminary analysis of the January-August results of the EU rapid testing program shows that in this sub-population BSE prevalence is, as an average for the whole E.U., approx. 15 times lower than in risk stock. This ratio is a rough guide which shows that to detect the same number of BSE positives as would be found by testing the risk populations, a far higher number of healthy stock need to be tested such as 100.000 risk stock but 1.5 million healthy stock.

b) **Ruminant species and sample size.** The question may be raised whether the calculated sample size should be different for cattle, sheep or goats. The choice of critical prevalence (p_o) and desired confidence ($1- \alpha$) may very well depend on species and be different for bovines, ovines and caprines These values can not be derived from statistical theory only but should result from considering:

- the consequences that non-detection of a higher true prevalence would have;
- *a priori* plausible prevalence;

- available risk management options.

It should also be noted that TSE prevalence in small ruminants may be very low (e.g., 10-50 per million) and that BSE, if it would occur in small ruminants, would only be a part, which is unknown, of total TSE prevalence. The likelihood of an animal being scrapie-infected will also depend upon its genotype. For UK, where TSE prevalence in small ruminants may be 500 per million, the ovine sample size calculations are outlined elsewhere in this report, together with the rationale for their derivation.

c) Technical-statistical issues.

- It may be noted that, because of the high numbers involved and the small values of p_0 (p_0<<0.10), the results of the above calculated sample sizes show hardly any difference whether derived from binomial or Poisson distribution.

  Note: more complicated models could be developed should details be available for some characteristics (e.g., age distribution, region). However, the Working Group expects that they would complicate the calculations but most likely not result in simplified sampling strategies nor in smaller samples.

- For smaller countries the number of fallen stock, emergency slaughter and sick slaughter to be tested may exceed the expected annual numbers of animals in this sub-population.

  It should be noted that the above calculated sample sizes are meant to be applicable for an animal population as an entity and not for the total number of animals present during a given year. The indicated prevalence values p_0 are *mean* proportions for a target (cattle) population (which can be a clearly defined regional or other sub-population of the whole (cattle) population of a country). The populations of animals born before and after the full implementation of a feedban should constitute two separate sub-populations, to be considered separately. In practice, variations may occur between regions, farm types, breeds, etc. within a same country. To achieve representativeness any sampling should therefore be taken at random from the whole target population.

  For countries with a large national herd, the required number of samples can be tested within a given calendar year. However, it is not excluded that for relatively small national herds the total sample size exceeds the number of animals that can be sampled during a given year. Therefore smaller countries may need several years to achieve the required precision of estimation, or for the upper 95% confidence limit to exclude a threshold prevalence. For large countries, the upper limit of the (1-α)x100 % confidence interval to estimate the true prevalence will gradually decrease (e.g., from 1/10.000 to 1/100.000) according as years with negative results succeed each other. However, for smaller countries [= if more than one year is needed to reach the required sample size], the number of successive years over which the sample can be cumulated will be limited by the length of the incubation period of the disease, in practice 4-6 years. It is only by the end of that period that the effects of (the absence of) risk management measures can be fully assessed.

The finding of positive test results will more easily be converted into precisely quantified prevalence figures in countries with a large national herd. But in smaller countries several years will be needed. In the latter case, the positive case(s) found in the sample analyzed during one given year will result in a high prevalence estimate (but also with high imprecision) both of which may reduce as data accumulate for the period over which prevalence is being estimated.

What precedes implies that the geographical status of "statistical" BSE freedom can more rapidly be reached in countries with a large national herd (or flock). However, the outcome of a survey with negative test results should also be evaluated / interpreted in the light of other information such as whether or not past risk management measures support the conclusion that a country is free of BSE (-risk).

- Other points to consider are sensitivity and specificity of the chosen diagnostic test, sensitivity being the conditional probability the test will give a positive result for a positive animal, specificity being the conditional probability the test will give a negative result for a negative animal. Both test characteristics depend on the tested animal's state of incubation.

d) Stratification

BSE is considered to be a disease that is not homogeneously distributed in space nor in time. Regional variations may occur within a country (e.g., Western France, southern Germany and Scotland as compared to the rest of the UK), there is a long incubation period (mean of 5 years from infection) and the number of cases will be significantly different before or after major risk management measures (e.g., a feed ban).

Questions may then be raised such as whether independent sampling schemes should be applied if a priori knowledge shows that there are important regional or time variations, whether a regional stratification can be applied in practice if it is only known that regional variations are very likely to exist but not to what extent, whether the sampling rates at a national scale would permit the results to be interpreted region-wise, etc.

The basic principle for developing sampling schemes considered in this text is that of random sampling from a clearly defined target population and results should be interpreted with reference to this defined target population. Random sampling is a way to ensure representativeness of the sample for the target population. Doing this minimises deviations of the sample compositions (with regard to, for instance, age classes or regions) from the population structure.

This means that if the sampling scheme was set up for the national herd (with a random sample from the national herd), the results should be interpreted at the national scale. A region-wise look within these results implies region-wise smaller sample sizes that correspond to either lower confidence in detecting a certain prevalence (now understood region-wise), or a higher prevalence that can be excluded, or a combination of both. The interpretation on the national scale and the region-wise interpretation of results from a specific sample have thus different quality and different reliability.

'Stratified sampling' can serve different purposes. If knowledge about certain structures in the target population (about age-wise or regional distribution) is available the sample needs to reflect this (ensuring 'more' representativeness), then the whole sample could be split or stratified according to the population structure.

For example: it is decided to sample 50,000 animals from the target population [say, UK's 800,000 OTMS bovines born before 1 August 1996] and it is known or estimated that the target population consists of 60% animals of age-class 1, 30% of age-class 2, and 10% of age-class 3 then an age-stratified sample, which applied the same sampling fraction [1/16] per age-class, would be expected to consist of 30,000 cattle from age-class 1 [1/16 * {800,000 * 60%} = 1/16 * 480,000], 15,000 from age-class 2 [1/16 * {800,000 * 30%} = 1/16 * 240,000] and 5,000 from age-class 3.

However, if the age-stratified sample was designed by applying the same sampling fraction in age-classes 1 and 2 but over-sampling from age-class 3 to ensure that the sample included 10,000 bovines from age-class 3, then its random sampling fraction would be 1/8 of 80,000 age-class 3 bovines. To yield an age-representative sample of 40,000 from the remaining 720,000 age-class 1+2 bovines, the sampling fraction has to be 1/18 in each of age-classes 1 and 2 to give 26,667 cattle from age-class 1 [1/18 * {800,000 * 60%} = 1/18 * 480,000] and 13,333 cattle from age-class 2 [1/18 * {800,000 * 30%} = 1/18 * 240,000]. This second example underlines why it is vital to describe fully any random sampling scheme, especially so because there may be good precision-related reasons for over-sampling in a particular age-class but for data to be comparable between countries which did (and did not) over-sample the sampling fractions [and results] per age-class need to be declared so that re-weighting to a common basis is possible.

Or if the 'stratification' is based on knowledge about prevalence of disease in different subgroups of the target population (sub-grouping by age or region for instance) then it would mean that separate sampling schemes for these subgroups (or sub-populations) need to be set up, specifying critical prevalences to be detected and required confidence levels separately for each subgroup. Interpretation of results would then refer to these regional or other sub-groups as well.

If a constant pattern of disease occurrence by age class were generally accepted, for example: there are three age-classes, age-class 2 has always the highest prevalence, prevalences in age-class 1 are always about 50% of those in age-class 2, and prevalences in age-class 3 are always about 30% of those in age-class 2, then it could be sufficient to sample from only one of those age-classes and predict prevalences for the rest based on this result. And that age-class for which the critical prevalence (the prevalence that should be detected) has the highest value (age-class 2 in the example) should be chosen as this would require the smallest sample size. But, such a strategy requires reliable knowledge of the pattern of disease and it requires this pattern to remain so in the future. For the moment, the available knowledge base is not sufficiently secure to raise the BSE testing lower age above 24 months.

V.6. SUMMARY

Rapid BSE test surveillance of apparently healthy bovines presented for slaughter and of risk

bovines has been implemented, increasingly comprehensively, in all Member States.

If nil / n tested bovines have been found BSE test positive, upper 95% confidence limit for BSE positivity should be taken as 3.7/N.

For most Member States (but not necessarily for all countries world-wide), the goal of rapid BSE test surveillance per target population is precise estimation of BSE prevalence.

Overall, BSE prevalence appears to be roughly 10-15 times higher in risk stock than in apparently healthy 30+ months bovines.

Active BSE surveillance of risk stock constitutes a BSE early warning; but there should also be sufficient active BSE surveillance at slaughterhouses to guard against diversion of BSE suspects.

To take account of differential implementation of rapid BSE test surveillance, a BSE reporting supplement is recommended from 2000 whereby Member States report their 'coverage-adjusted' estimated BSE cases per calendar year.

Across Member States, age distribution of BSE rapid test positive a) apparently healthy stock and b) risk stock and c) clinical BSE cases need to be formally compared.

When EU standard is rapid BSE testing in apparently healthy stock aged 30+ months, Member States choosing to extend rapid BSE testing to younger animals should nevertheless ensure that they can specifically report their test results [numerator and denominator] for bovines aged 30+ months. Testing at younger ages otherwise increases the denominator disproportionately to the numerator compared to other countries.

Rapid TSE testing of native adult small ruminants presented for slaughter should include genotyping [and flock identification] of a random 0.5% of the first 100,000 native adult sheep subject to rapid TSE testing per Member State.

In addition, genotyping and flock identification should be undertaken for each TSE rapid test positive adult sheep and its associated 6 controls [3 slaughterhouse controls + 3 within-flock controls].

To exclude a BSE prevalence in TSE rapid test positive adult sheep of 1 in 200 TSE test positives, a Member State would need to apply second-stage BSE testing to between 600 and 920 TSE rapid test positives without finding any TSE positive animal which is BSE positive.

VI. LITERATURE

Cannon, R.M., Roe, R.T., 1982. Livestock Disease Surveys: a Field Manual for Veterinarians. Australian Bureau of Animal Health, Canberra, 34 pp

E.C. (European Commission, 1999). The risks of non conventional transmissible agents, conventional infectious agents or other hazards such as toxic substances entering the human food or animal feed chains via raw material from fallen stock and dead animals or via condemned materials. Report prepared by the TSE/BSE *ad hoc* Group for the Scientific Steering Committee (adopted on 25.06.99).

Hart, R.J., Church, P.N., Kempster; A.J., 1997. Audit of bovine and ovine slaughter and by-products sector (Ruminant Products Audit). Prepared for MAFF. 42 pages + annexes.

VII. ACKNOWLEDGEMENTS

The Scientific Steering Committee and its TSE/BSE *ad hoc* Group acknowledge the scientists who have contributed to the present report: Dr. S.Bird (rapporteur), Dr.D.Heim, Dr. S.Dahms, Prof.Dr.R.Palm, Dr.J.Delincé.

ANNEX : TSE SURVEILLANCE: COMMENTS ON POPULATION AND SAMPLE, STRATIFICATION AND TEMPORAL EVOLUTION OF BSE PREVALENCE[18].

1. Population and sample

One of the most difficult points seems to be the concept of population.

To be more specific, one can for example consider the case of Luxembourg (the figures given here are not necessarily accurate but serve as an illustration).

1.1. The **number of bovine animals aged over two years** is around 100 000. These 100 000 bovines constitute a population in terms of National Statistics. Assuming, for example, that in a given year (e.g., the year 2000) 1 500 animals were sent for emergency slaughter (casualty stock), these animals constitute a sub-population in terms of National Statistics. The terms population and sub-population refer in this case to sets of animals which exist in reality.

1.2. The notion of **BSE prevalence among the emergency slaughter animals.** To simplify matters, one can first of all assume that this prevalence, represented by the symbol "p", does not change over time. If there are no positive animals among the 1 500 emergency slaughter animals tested, this offers no guarantee that the Luxembourg "system of production" is free of the disease because a positive animal will perhaps be detected tomorrow. This clearly demonstrates that the prevalence one is concerned with is not the prevalence in the real herd of 1 500 emergency slaughter animals, but is the prevalence associated with a method of cattle production and the 1 500 emergency slaughter animals constitute a sample of a theoretical population of animals (i.e. which does not exist in reality) which might have been or which might be sent for emergency slaughter in the future. This is the theoretical population referred to in the main report). In this context, animals actually sent for emergency slaughter during a given period, which is not necessary equivalent to a year, constitute a sample. Nothing in fact prevents from cumulating emergency slaughter animals over a period of more then 12 months if one wishes to have a sample exceeding 1 500.

The sample in question may, from a statistical point of view, be treated as a simple random sample, even though, in practice, no sampling operation takes place, strictly speaking.

The above point is fundamental, since sample size is determined exclusively on this basis: the population of interest is not the sub-population of 1,500 animals sent for emergency slaughter during a specific year. The period of one year is, moreover, purely arbitrary: why should one take a period of one year as a reference period rather than a half-year or quarter?

[18] Annex report prepared by Prof.Dr.R.Palm, Unité de Statistique et Informatique, Faculté universitaire des Sciences Agronomiques, B-5030 Gembloux , BELGIUM

2. Stratification

Assuming an *a priori* knowledge, on the basis of previous studies, that emergency slaughter cattle aged from 24 to 30 months have a prevalence equivalent to p_1 and that those aged over 30 months have a prevalence equivalent to p_2 (p_2 different from p_1) and assuming that the proportions of cattle belonging to each of these two categories are constant over time and are equal, respectively, to W_1 and W_2 then the prevalence for both categories is equal to: $p = W_1 p_1 + W_2 p_2$, with $W_1 + W_2 = 1$.

In this context, two different problems may arise:

a) One is interested in p_1 and p_2 and not just p;
b) One is interested only in p.

If the inference (hypothesis testing or upper confidence limit) relates both to p_1 and p_2, the two age categories are dealt with separately. Each category then corresponds to a population and the total number of populations is simply multiplied by two for the EU. The sample sizes calculated in the main report are then the numbers of animals necessary in each category.

If the statistical inference relates only to p, one may ask whether it is better to stratify into two categories, take a sample per category, and then combine the results of the two samples. The answer is that there isn't much point in taking stratification into account. In other words, for a given degree of confidence, the number of observations to be made in each category ($n_1 + n_2$) is the same as the number (n) of observations to be made in the absence of stratification. It is of course assumed that sampling remains simple and random: a country which removed animals belonging to the category with the higher prevalence would obviously falsify the result.

The above remarks made concerning the two age categories may be generally applied to other stratification criteria (region of a country, for example): stratification may be performed, but it offers no appreciable benefits in terms of the total number of animals to be examined.

Stratification is thus necessary if one requires detailed information relating to the sub-groups. Otherwise, is not essential except if the sampling rates are adapted per stratums (ee main report).

3. Population trend over time

It has been shown above that emergency slaughter animals could be "cumulated" over time in order to obtain a sufficiently large sample. This poses no specific problems as long as the prevalence p remains stable over time. The situation is more complex when the prevalence changes over time.

If, for example, one uses the information obtained from animals sent for emergency slaughter over a two-year period, the prevalence p, to which the statistical inference relates, must be considered as the average prevalence during the two-year observation period.

The problem may be considered to be less serious if prevalence decreases over time. In fact, if the average prevalence p for the period is considered less than a critical prevalence p_0, *a*

fortiori the prevalence at the end of the period, which is less than p, will be considered as lower than p_0.

If there is a substantial change in prevalence over time, one may, however, question the use of an inference relating to the average prevalence during the period: what is the use of knowing that the average prevalence over a period of time is less than p_0, if we know that the real prevalence at the end of the period is very different from the average prevalence?

There seems to be no simple solution for taking into account data collected over a long period if the inference relates only to the prevalence at the end of the period.