

**Report of the
Task Force on Zoonoses Data Collection
on a proposal for technical specifications for a
baseline survey on the prevalence of *Salmonella* in
breeding pigs¹**

(Question N° EFSA-Q-2006-044)

**Adopted by
The Task Force on 30 April 2007**

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Summary

Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents lays down provisions for the control of *Salmonella* and other specified food-borne agents. The provisions require the setting of reduction targets for *Salmonella* in live pigs within a fixed time schedule. Pursuant to the provisions, a Community target should be established for the reduction of the prevalence of *Salmonella* in breeding herds of pigs at primary production. The European Food Safety Authority (EFSA) was asked by the European Commission to prepare a proposal for the technical specifications of a baseline survey of the prevalence of *Salmonella* in breeding pigs in the European Union.

The proposal presented focuses on the estimation of the *Salmonella* prevalence in holdings having breeding pigs infected with *Salmonella*. The prevalence is measured separately in breeding holdings and production holdings in the European Union and in the Member States. The data from the breeding holdings will give information on the infection level of *Salmonella* in breeding pigs that are sold for breeding purposes to production holdings, while the survey undertaken in production holdings will provide information on the exposure to *Salmonella* of fattening pigs by breeding pigs.

A study of within-holding prevalence of *Salmonella* provides further information, which can be used to adjust the Member State specific prevalence figures.

Standardised EN/ISO methods are proposed to be employed in the laboratory analyses of samples for detection of *Salmonella*.

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A. Background and terms of reference

Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents¹ lays down provisions for the control of *Salmonella* and other specified food-borne agents. The scope of the Regulation is limited to zoonotic agents which pose a public health concern. The Regulation foresees the setting of Community targets for the reduction of *Salmonella* at the level of primary production, and where appropriate, at other stages of the food chain. Target setting in poultry populations and slaughter pigs is ongoing. Pursuant to the Regulation, a Community target should also be established for the reduction of the prevalence of *Salmonella* in breeding herds of pigs at primary production.

In order to set the target, comparable data on the prevalence of *Salmonella* in breeding herds of pigs in the Member States (MSs) should be available. Therefore, a special survey must be carried out in order to estimate the prevalence of breeding herds of pigs that are infected with *Salmonella*. The Community baseline surveys are coordinated by the European Commission. With the aim of launching a baseline survey to estimate the prevalence of *Salmonella* in breeding herds of pigs at European Union level and in the MSs, the Commission has requested the European Food Safety Authority (EFSA), on 28 April 2006, to prepare technical specifications for such a baseline survey. EFSA assigned this task to the Task Force on Zoonoses Data Collection, and a specific Working Group was set under the Task Force to prepare a draft report on the subject.

The mandate was to prepare a proposal for technical specifications for a baseline survey of the *Salmonella* prevalence in breeding herds of pigs. This scheme should be designed as a survey protocol and cover, in particular:

- the delineation of the population to be sampled,
- the data to be collected,
- the case definitions,
- the sampling scheme,
- the analytical methods,
- and the reporting.

Furthermore, the primary and secondary addressed questions of the baseline survey, i.e. the prevalence parameters and the risk factors, should be stated.

¹ Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents. *Official Journal of the European Union* 2003; L 325/1: 12.12.2003.

B. Rationale for the choices made in the proposal

While preparing the technical specifications for the baseline survey of *Salmonella* in breeding pigs, some decisions and choices regarding the scope and design of the specifications were made. These decisions and their rationales are described below.

1. The choice of sampling the holdings

It is suggested that the breeding pigs are sampled at the holding level. It is important to emphasise that the survey is not intended to estimate the prevalence of infection within each holding but rather to estimate the prevalence of infected holdings. The rationale for this is as follows:

- Although breeding animals do enter the food chain at the end of their productive lives, such animals are a minor contribution to the risk of *Salmonella* infections in humans. Therefore sampling at the slaughterhouse level was not chosen.
- Breeding pigs may play an important role in the maintenance and transmission of *Salmonella* infection either to the slaughter generation (production herds), or act as a source of infection to the breeding pigs whose progeny will become the slaughter generation (nucleus and multiplier herds).
- Thus, the purpose of this survey is to estimate the prevalence of holdings with breeding pigs infected with *Salmonella* that represent a risk to other pigs – either breeding herds lower down the pyramid or directly to slaughter pigs. For pragmatic reasons only, holdings with a 10% or higher within-holding prevalence will be detected with a 95% confidence.
- It is considered that the overall status of the holding with breeding pigs – either positive or negative – rather than the within-holding prevalence is the most important value to estimate. Herd status is likely to be more constant than within-herd prevalence. The number of infected and excreting pigs within a herd may be subject to variation, for instance in response to the season, stress, inter-current disease, or stage of production. Adult breeding pigs may live for four years or more and produce perhaps 80 or more progeny in their lifetime. Thus, there is considerable potential for infection to be disseminated through their progeny and this risk may be most effectively mitigated through actions at the holding level. Furthermore, since interventions are at a holding level, success can be defined as a decrease in the proportion of ‘positive’ holdings with breeding pigs.
- There is an important clustering effect in holdings, so that an individual pig is much more similar to other pigs on the same holding than pigs on another holding. Thus, the holding level information reflects the general status of the pigs within that holding.
- Estimation of holding prevalence implies that herd size is not taken into account. Prevalence is a proportion, in which the numerator is the number of positive results and the denominator is the number of units tested. A holding is classified as positive if at least one pooled sample is positive, and negative if all samples are negative. Thus, one very large holding with *Salmonella* represents one positive result in the numerator and a very small herd with *Salmonella* also represents one positive result in estimation of the herd prevalence. However, the actual number of individual pigs that are infected is likely to be greater in the larger holdings. Furthermore, larger holdings have a greater risk of being *Salmonella* positive, and there is considerable variation in the distribution of herd size amongst MSs. Sampling at animal level, to estimate the overall prevalence of infection amongst individual pigs, would eliminate the problem of differences in herd sizes both

within, and between, MSs. If baseline studies are repeated to determine whether prevalence has changed, then comparison at the animal level would be easier to interpret than at herd level. However, despite conducting the survey to estimate a holding prevalence estimate, it will be possible to estimate a predicted animal level prevalence, provided that adequate sample level data are recorded, and that the sensitivity of the sampling method is known, as proposed in the within-holding prevalence study. The distribution of holding sizes is skewed and it can be expected that a few of the largest holdings will contain a significant proportion of all breeding pigs. It is important that these holdings are represented in the survey, and this is achieved by stratification according to the holding size. The number of sampled holdings in each stratum should be proportional to the total number of holdings with breeding pigs in each stratum.

2. Rationale for the use of the terms ‘holdings’ as opposed to ‘herds’

The definition of a herd may vary in different MSs. In some circumstances, there may be considered to be more than one herd in one holding, whilst in others, the pigs that are considered to comprise one herd may be distributed across more than one holding. Thus, herd was not a term sufficiently precise to be used as a definition of primary sampling unit. The option of considering other epidemiological units (e.g. breeding pigs) was considered to be prone to misinterpretation. Therefore, it is recommended that sampling shall be done at holding level, as holding is a legally defined entity in all MSs, and therefore the interpretation of ‘holding’ should be the same in all MSs. According to EU-regulations, all pig holdings must have a unique identity with a unique geographic location.

3. Choice of the delineation of the population

The aim of the survey is to make inferences to the population of holdings harbouring at least 80% of the breeding pig population in the MS.

In some MSs there are many small holdings (with less than 10-15 breeding pigs) which only represent a relatively small part of the whole breeding pig population. These holdings are not likely to be typical of the population of holdings with breeding pigs and they are unlikely to represent an important risk of either transmission of *Salmonella* to other holdings or to public health. They may, however, be important in some niche areas such as rare breeds or specialist local goods. The present survey is not designed to measure prevalence in such smaller populations. To avoid circumstances where that the estimated holding prevalence might not be a useful indicator of risk to either other pigs or people since it would contain many small holdings, it was decided that when possible, holdings to be sampled should have at least 50 breeding pigs. However, since the holdings in the sampling frame must include at least 80% of all breeding pigs in the MS, it is recognised that in some MSs holdings with less than 50 breeding pigs may have to be included to fulfil this requirement.

Holdings with breeding pigs may be classified as breeding holdings or as production holdings. The distinction between these two types of holding is that breeding holdings sell breeding pigs, whereas production holdings only sell pigs for fattening (either rearing them to slaughter weight on the holding on which they were born or on another holding). These two groups of holdings with breeding pigs can be further classified as:

1. breeding holdings:

- a. nucleus holdings (main objective is to sell purebred boars or their semen and gilts (at least 40%) to multiplier holdings and piglet producing holdings),

- b. multiplier or supplier holdings (main objective is to sell (crossbreed) gilts (at least 40% of gilts) to production holdings (farrow to weaner and farrow to finish holdings),
2. production holdings:
- c. farrow to weaner holdings (piglet producing holdings) or farrow to grower where the progeny will be reared for slaughter on another holding,
 - d. farrow to finish holdings.

Based on this classification, it is proposed to carry out one survey in breeding holdings and another one in production holdings. Although the finisher pigs from farrow to finish holdings have been surveyed at a national level in the framework of the 'Baseline survey on the prevalence of *Salmonella* in slaughter pigs in the EU'¹, neither the herd level infection nor the breeding pigs within these herds were investigated in this survey. For this reason, and to ensure a full set of representative data to support the *Salmonella* control programmes in pigs and for future risk analysis, it is proposed to consider that breeding pig population in the two groups of holdings with breeding pigs.

4. Rationale for conducting two surveys, one in breeding holdings and one in production holdings

The breeding holdings are of special importance for *Salmonella* infections in pigs. They are at a crucial position at the top of the production pyramid and thus have a unique potential role in the dissemination of *Salmonella* infection throughout the whole production chain if the holdings are infected. It is therefore important that these holdings be given special consideration.

Since in most countries only a small minority of holdings are breeding holdings it is probable that only few of them would be selected in a survey covering all holdings of breeding pigs. Therefore an own survey for breeding holdings is proposed to guarantee a sample sufficiently large of these holdings in the base-line survey to enable to estimation of the *Salmonella* prevalence specifically in this holding category.

5. Rationale for the data collection for the risk factor analysis

In order to investigate major risk factors, the survey will afford the opportunity to collect data on holding-level variables that may be associated with the *Salmonella* status of the holding. However, sample size calculations have not been predicated on this secondary aim. In addition to estimating the prevalence of holdings with breeding pigs infected with *Salmonella*, the survey data may be used to undertake an analysis at the level of sample. An important holding-level clustering effect is expected, such that pens or groups of pigs on one particular holding are much more alike than another randomly selected pen or group on some other holding. This clustering effect will reduce the power of the study to detect statistically significant associations. Nonetheless, such an analysis may provide useful input data for preparation of control programmes as well as future risk assessments.

¹ Commission Decision of 29 September 2006 concerning a financial contribution from the Community towards a baseline survey on the prevalence of *Salmonella* in slaughter pigs to be carried out in the Member States. 2006/668/EC. *Official Journal of the European Union* 2004; L275/51: 6.10.2006.

6. Primary sample size calculation (number of holdings to sample)

There is no information available on *Salmonella* prevalence that provides an estimate of the likely prevalences for each of the MSs, and thus it was decided to use a 50% assumed prevalence (worst case scenario with highest sample size).

Sample size calculation tables are presented for several options of precision and for varying numbers of populations of holdings with breeding pigs. Both the accuracy levels of 5% and 7.5% are assumed to provide sufficient data for the prevalence estimates at the EU level.

7. Secondary sample size calculation (within-holding number of samples) and rationale for the pooling and estimation of herd-level *Salmonella* status

This rationale is described in the Annex I and it is based on the data and analyses provided by the United Kingdom.

8. Rationale for proposing the investigation of the *Salmonella* within-holding prevalence

It is important to note that the field and experimental data underlying the pen sampling approach derives from the United Kingdom (UK) alone. It is possible that the range of values applicable to the parameters in the model varies importantly in other MSs and the sensitivity of the sampling strategy may thus differ importantly from the 10% within-holding prevalence based on the UK data.

Many variables that could influence the probability of isolating *Salmonella* from a pooled sample may vary systematically amongst the MSs. Examples of such variables are husbandry systems, group sizes, dietary components, background environmental exposures, and disease status.

It would be preferable to base the prevalence estimates on same within-holding prevalence sensitivity in all the MSs. For this purpose, it is possible to adjust individual MS' *Salmonella* holding prevalence estimates using the results from a limited number of more intensively sampled holdings (within-holding prevalence study).

Therefore, it is recommended that 10 holdings, selected at random from the sample list, are subjected to more intensive sampling to investigate the within-holding prevalence. At least 10 fresh individual faecal samples will be collected from each pen or group. Each sample will be divided into 2 parts in the laboratory. One part will be cultured individually, whilst the second part will be used to prepare a pooled sample.

These data will provide the necessary information to revise the predicted sensitivity of the pooled pen sampling and enable the MSs-specific holding level prevalence to be adjusted. The data may also be used to investigate the systematic differences amongst MSs described above.

There are different options to conduct the within-holding prevalence study. However, the mandatory one would be the preferred option from the scientific point of view:

Mandatory option

In the case of a mandatory implementation of the study, the holding prevalence estimates could be adjusted in the MSs based on their own within-holding prevalence data, when deemed necessary. This would provide the most accurate estimations of the *Salmonella* holding prevalence throughout the EU. Fully comparable MSs prevalence figures would be available.

Voluntary option

If the within-holding prevalence study would be carried out only by some MSs, the prevalence figures between MSs would be less comparable since they could be based on different within-holding prevalence cut-offs. The data from the survey would be more difficult to interpret and the Community *Salmonella* holding prevalence would be less accurate. Nevertheless the within-holding prevalence data derived from some MSs could be used to adjust the holding prevalence estimates of all the MSs based on an assumption that there are similarities between the MSs. It is important that any MS choosing not to implement the within-holding prevalence study would need to accept that data derived from other MSs would be used to adjust their prevalence figures.

Option: no within-holding prevalence study

The consequences of not carrying out the study would be that no data are collected to reliably estimate the within-holding prevalence. Consequently, a *Salmonella* holding prevalence would be estimated without a proper idea of the accuracy of declaring holdings *Salmonella* positive or negative. This would impact the reproducibility of the survey, which might be required to determine whether MSs achieved a future target for reduction.

9. Rationale for the approach regarding phage typing and antimicrobial susceptibility testing

Characterisation of isolated strains by serotyping is obligatory. It is, however, recommended that selected strains are further characterised as this will add useful and important information to the results. The additional results may be used for epidemiological analysis and for characterisation of the risk of spread of antimicrobial resistance. Strategy and methods of antimicrobial susceptibility testing should follow the guidelines already given in the “Report including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys and pigs and *Campylobacter jejuni* and *C. coli* in broilers¹.”

¹ Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers, *The EFSA Journal* (2007), 96, 1-46.

C. Proposed technical specifications for a baseline survey on the prevalence of *Salmonella* in breeding pigs

1. Introduction

Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents¹ lays down provisions for the control of *Salmonella* and other specified food-borne agents. The Regulation foresees the setting of Community targets for the reduction of *Salmonella* at the level of primary production. Pursuant to the provisions, a Community target should be established for the reduction of the prevalence of *Salmonella* in breeding herds of pigs at primary production.

In order to provide the scientific basis for setting of the Community target for reduction of the prevalence of *Salmonella* in breeding herds of pigs, a European Union-wide *Salmonella* baseline study should be carried out.

In these technical specifications, the baseline study takes the form of a one year baseline survey.

Before the initiation of the survey, MSs are encouraged to organize training for the involved parties.

2. Definitions

For the purpose of this document, the following definitions will apply. General epidemiological definitions (like population, prevalence, random sampling, precision, sample, sampling frame, sampling units and others) can be found in EFSA's Report on Guidance on Good Practices for Design of Field Surveys².

Breeding holding

Means holding having pigs retained for breeding purposes and it covers nucleus holdings and multiplier holdings. Breeding holdings produce and sell pigs for breeding purposes.

Breeding pig

Means a pig (sow or boar) of at least six months of age kept for breeding purposes.

Farrow to finish holding

Means a holding of animals that are included in a production system that contains all production stages, from breeding to gestation to farrowing to nursery to grow-finishing to market.

¹ Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *salmonella* and other specified food-borne zoonotic agents. *Official Journal of the European Union* 2003; L 325/1: 12.12.2003.

² Report of Task Force on Zoonoses Data Collection on Guidance Document on Good Practices for Design of Field Surveys, *The EFSA Journal* (2006), 93, 1-24.

Farrow to weaner holding, farrow to grower holding

Means a holding of animals that are included in a production system where pigs are sold out of the weaner or nursery phase (approximate age 11 weeks) or as grower pigs (approximate age 23 weeks) to a finishing operation to grow them to market weight.

Fattening (finisher) pig holding

Means a holding of feeder pigs that are fed until they reach the market weight.

Herd

Means an animal or group of animals kept on a holding as an epidemiological unit (Regulation (EC) N°. 2160/2003). For the purpose of this baseline survey, 'herd' is considered equivalent to 'holding'. The survey will make reference to the term "herd" as epidemiological unit, in order to be coherent with the objectives established in Regulation (EC) No 2160/2003, *i.e.* to set a Community target for the reduction of the prevalence of *Salmonella* in breeding herds of pigs.

Holding

Means any establishment, construction or, in the case of an open-air farm, any place in which animals are held, kept or handled (Council Directive 92/102/EEC).

Multiplier holding (syn. supplier holding)

Means a holding of purebred animals that produces, as the main commercial objective, (usually crossbred) first generation breeding animals for production holdings.

Nucleus holding (syn. purebred holding)

Means a holding of purebred animals that produces purebred breeding animals (or purebred females and / or boars) to the multipliers and commercial (production) holdings.

Positive for *Salmonella*, Holding

Holdings where at least one pooled faeces sample taken was positive for any *Salmonella*, irrespective of the serovar identified.

Positive for *Salmonella* serovar, Holding

Holdings positive for the *Salmonella* serovar specified.

Production holding

Means a farrow to finish holding, a farrow to weaner holding, or a farrow to grower holdings. Production holdings sell pigs only for fattening purposes (either rearing them to slaughter weight on the holding on which they were born or on another holding).

3. Objectives

3.1. Primary objective

The main objective of the survey is to establish the baseline prevalence of holdings with *Salmonella* infected breeding pigs in the Community and in the MSs. The survey-design is optimised according to this primary objective only.

Specifically, the survey shall collect sufficient samples from each selected holding to enable such holding to be classified as follows:

- At least **one** sample is positive to *Salmonella* – holding is defined as positive, meaning that it has a within-holding breeding pigs *Salmonella* prevalence of 10% or higher;
- **All** samples are negative to *Salmonella* – holding is defined as negative, meaning that it has a within-holding breeding pigs *Salmonella* prevalence of less than 10%

This prevalence will be measured separately in breeding holdings, as well as in production holdings in the Community and in the MSs. The data from the breeding holdings will give information on the infection level of *Salmonella* in breeding pigs that are sold for breeding purposes to production holdings in each country, while the survey undertaken in production holdings will provide information on the exposure to *Salmonella* of fattening pigs by the breeding pigs.

Sufficient holdings will be selected within each MS to ensure that the prevalence of infected holdings in breeding and in production holdings can be estimated with a desired precision. The survey is designed so that the results shall be comparable amongst the MSs. The survey shall cover a one year period.

3.2. Secondary objectives

The survey will afford the opportunity to collect data on a restricted set of holding-level and sample-level variables that may be associated with *Salmonella* prevalence. However, sample size calculations have not been predicated on this secondary aim.

Moreover, on 10 holdings with breeding pigs in every MS a more intensive within-holding individual animal-level sampling study is proposed in order to validate the pooled sample strategy and to investigate the *Salmonella* within-holding prevalence. This is done to facilitate the adjustment of the MSs-specific prevalence figures to correspond to the same within-holding prevalence detection level, when necessary.

Lastly, *Salmonella* strains will be available for antibiotic susceptibility testing to give valuable information on the prevalence of antimicrobial resistance.

4. Sampling design

4.1. The delineation of the population

The aim of the survey is to make inferences to the population of holdings harbouring at least 80% of the breeding pig population in the Community and in the MSs. Holdings having 50 breeding pigs or more should be included in the sampling. However, if the holdings having 50 breeding pigs or more do not contain 80% of the national herd of breeding pigs, then smaller holdings with less than 50 breeding pigs should also be sampled. In case holdings having less than 50 breeding pigs are to be sampled, individual MSs may use a lower herd size cut-off that is appropriate for their own herd structure within their individual survey plans.

The *Salmonella* prevalence shall be measured separately in breeding holdings as well as in production holdings in the Community and in the MSs.

4.2. The sample and the sampling strategy

Both surveys have a similar two-stage sampling design. In a first stage, a sample of holdings is selected in every MS for both surveys. In the second stage, a number of pens are selected for sampling within every selected holding.

4.2.1. First stage: selection of holdings

In each MS, holdings with breeding pigs must be randomly selected from the sampling frame of breeding holdings and of production holdings separately.

The number of holdings to be sampled shall be stratified according to the number of holdings with breeding pigs in the different categories of holding size (number of breeding pigs of 1-49; 50-99; 100-399; 400-999 and > 999). If the obtained number of samples is not an integer, it shall be rounded (up) to the nearest integer. The holdings are selected by a random procedure from each stratum.

If a selected holding can not be sampled (for example, if it no longer exists when sampling should be done) a new holding is selected at random from the same stratum in the target population.

The primary sample size (number of holdings to be sampled) must be equally distributed over the year to cover the different seasons, as far as possible. To this end the 12-month period is to be divided in 12 periods of 1 month. One twelfth of the number of holdings is to be taken in each of those periods.

Outdoor holdings are to be included in the surveys, but there will be no stratification on this production type.

4.2.2. Second stage: selection of pens to be sampled

Secondly in the selected holdings the pens, yards or groups of breeding pigs (over 6 months of age) to be sampled are randomly chosen.

The number of pens, yards, or groups to be sampled must be proportionally allocated according to the numbers of breeding pigs in the different stages of production (pregnant, non-pregnant, and

other categories of breeding pigs). The exact age categories to be sampled will not be prescribed, but this information must be collected during the sampling.

Breeding pigs that have arrived recently to the herd and are held in quarantine are not to be included in the surveys.

4.3. The sample size (number of samples) calculation

4.3.1. Primary sample size (first-stage sample size)

A regular primary sample size calculation must be done in both surveys. The primary sample size is the number of holdings to be sampled per MS, and it is determined considering the following criteria, assuming simple random sampling:

- The total number of breeding holdings (breeding holdings survey) or the total number of production holdings (production holdings survey)
- Annual expected prevalence (**p**): 50%
- Desired confidence level (**Z**): 95%, corresponding to a Z_{α} value of 1.96
- Accuracy (**L**): 5% or 7.5%
- Using these values and the formula:
$$n_{\infty} = \frac{(Z_{\alpha})^2 p(1-p)}{L^2}$$

For an infinite population size of holdings (i.e. more than 100,000 units infinite¹) the sample size is 384 for 5% accuracy. For finite population sizes of holdings (i.e. less than 100,000 units) per MS, the sample size is smaller because a finite population correction factor is applied.

Primary sample-size calculation according to 50% expected prevalence and 5% and 7.5% accuracy levels is in Table 1.

4.3.2. Secondary sample size (second-stage sample size)

Together 10 pens, yards, or groups of breeding pigs should be sampled in each holding. One pooled faeces sample is taken from each selected pen, yard, or group. If necessary, a group can consist of more than one pen. At least 10 individual pigs must contribute to each pooled sample.

However, where on small holdings or holdings with large numbers of pigs kept outdoors in paddocks, the number of pens, yards or groups is less than 10, repeat sampling of the same pen, yard, or group is required so that a total of 10 pooled samples are submitted.

¹A population is considered infinite when it is above 100,000.

Table 1. Number of holdings with breeding pigs to sample according as a function of the finite population size (total number of holdings with breeding pigs in the Member States)

Number of holdings with breeding pigs (= N = finite population size)	approximate sample size n for finite population size level of confidence 95% 50% assumed prevalence		
	accuracy		% sample size reduction
	5%	7.5%	
1,000,000	384	171	55
100,000	383	171	55
10,000	370	169	54
5,000	357	166	54
2,000	323	158	51
1,000	278	147	47
500	218	128	41
250	152	102	33
150	108	80	26
125	95	73	23
100	80	64	20
90	73	59	19
80	67	55	18
70	60	50	17
60	52	45	13
50	45	39	13
40	37	33	11
30	28	26	7
20	20	18	10
10	10	10	0

5. Sample collection in the herds

5.1. Type and detail of sample

The material collected for bacteriological analysis will be freshly voided faeces representing the whole holding, which is the unit of interest. Since every holding is unique it should be decided, before starting the sampling, which pens, yards, or groups within the holding are sampled. The sample collected must be placed in a separate jar avoiding cross contamination and sent to the laboratory. Details on the within-holding sampling procedure are provided in Annex II.

Each pooled sample should total at least 25g and two approaches may be employed to collect these pooled faeces samples:

1. where there is an accumulation of mixed faeces within an area of a pen or yard, a large swab can be used to pass through the faecal mass, ensuring that at least 25g of mixed material is collected,
2. where there is no such accumulation, for example in a large yard, in a farrowing house, or pens or other accommodation with low numbers of pigs per group, then individual pinches should be selected from individual fresh faecal masses so that a minimum of 10 individuals contribute to a total sample volume of at least 25g.

Approach 1 is to be preferred where practical. In this approach at least 10 individual pigs must contribute to each sample taken, otherwise approach 2 is to be applied.

Sampling shall be performed by the competent authority or under its supervision, by bodies to which it has delegated this responsibility.

5.2. Additional sampling for the within-holding prevalence study

Together, 10 holdings, selected at random from the overall sample of breeding and production holdings are subjected to more intensive sampling.

On these holdings at least 10 fresh individual faecal samples of at least 30g will be collected from each pen or group that would usually have contributed a pooled sample. Each sample will be divided in the laboratory into 2 parts. One part (25g) will be cultured individually, whilst the second part will be used to prepare a pooled sample. It will be essential that the individual samples from each holding are uniquely identified and that the identity of each individual sample that has been added to a pool is also recorded.

5.3. Sample information

All relevant information available from the sample should be recorded on a sampling form produced by the competent authority to enable the data requirements in section 'Reporting' to be fulfilled.

Each sample and its sample form should be labelled with a unique number which should be used from sampling to testing. The competent authority must arrange for the issue and use of a unique numbering system.

5.4. Transport of samples

Samples should be preferably kept at between +2 to +8°C and free of external contamination during transportation. The samples should be sent to the laboratory as quick as possible within 36 hours by fast mail or courier and shall reach the laboratory no later than 72 hours after sampling.

6. Laboratory analytical methods

6.1. Laboratories

Analysis and serotyping shall take place at the National Reference Laboratory (NRL). In case that the NRL does not have the capacity to perform all the analyses or if it is not the laboratory that performs detection routinely, the competent authorities may decide to designate a limited number of other laboratories involved in official control of *Salmonella* to perform the analyses. These laboratories should have proven experience of using the required detection method, and have a quality assurance system complying with ISO standard 17025 and be submitted to the supervision of the NRL.

6.2. Receipt of samples

Samples arriving after 72 hours after sampling shall be discarded unless analysis is initiated within 96 hours after sampling and the cold chain was not interrupted.

At the laboratory, samples shall be kept refrigerated until bacteriological examination, which shall be carried out within 24 hours after receipt and so that analysis is initiated no later than 96 hours after the sample was collected.

6.3. Sample analysis

6.3.1. Preparation of the specimen

In the laboratory, pen samples are homogenized in a stomacher before 25g is collected for analysis.

For evaluation of the pooling strategy, each of the individual collected samples (30g) needs to be divided into 2 parts. One part, weighing at least 25g is homogenised in a stomacher and subsequently cultured individually. The remaining second part will be used to prepare the pooled sample. The latter is prepared by adding 10 times 2.5 g of the individual samples to a pool similar to the routine pen sample of 25g. The pooled samples are homogenised in a stomacher before analysis.

6.3.2. Detection and identification methods

Detection of *Salmonella*

The method recommended by the Community Reference Laboratory (CRL) for *Salmonella* in Bilthoven, The Netherlands, shall be used. This method is described in Annex D of ISO 6579, 2006: 'Detection of *Salmonella* spp. in animal faeces and in samples of the primary production stage'.

Serotyping of *Salmonella*

All strains isolated and confirmed as *Salmonella* spp. shall be serotyped according to the Kaufmann-White scheme, by the National Reference Laboratory for *Salmonella*.

For quality assurance, 16 typable strains and 16 non-typable isolates shall be sent to the Community Reference Laboratory (CRL) for *Salmonella*, with a maximum of 16 non-typeable isolates. A proportion of these isolates should be sent to the CRL on a quarterly basis. If fewer strains have been isolated, all shall be sent.

Phage typing of *Salmonella*

For *S. Enteritidis* and *S. Typhimurium* it is recommended that at least one isolate from each positive sample should be phage typed, using the protocol defined by Health Protection Agency (HPA) Colindale, London.

6.4. Storage of isolates

In order to allow, for instance, later testing for antimicrobial susceptibility, of the isolates storage of a representative subset of isolates is obligatory. Only one isolate per serovar per positive holding shall be stored. Together at least 170 randomly selected isolates of *Salmonella* should be stored. However, if the total number of the available isolates is less than 170, all the isolates (one isolate per serovar per positive holding) shall be stored. Records of bacteriological analyses shall be kept on all samples processed.

All strains isolated shall be stored at the National Reference Laboratories (NRLs) using the normal method for NRL culture collection, as long as it ensures viability of the strains for a minimum of 5 years. In particular, strains should be available for monitoring on antimicrobial resistance according to Article 7 of Directive 2003/99/EC.

6.5. Testing of antimicrobial susceptibility

It is proposed that *Salmonella* isolates are tested for antimicrobial susceptibility according to the guidelines in the Report, including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs, and *Campylobacter jejuni* and *C. coli* in broilers¹.

The reporting of the antimicrobial susceptibility testing and phage type data is proposed to be done in accordance with Article 9 of Directive 2003/99/EC (annual national report on zoonoses).

7. Reporting from the Member States

7.1. General provisions

The competent authority responsible for the preparation of the yearly national report on zoonoses pursuant to Article 9 of Directive 2003/99/EC shall collect and evaluate the results and report them to the Commission.

The Commission shall forward the result to the European Food Safety Authority, which shall examine and report on them. Any use of the data submitted by the MSs for the purposes other than the objective of this survey will be subject to prior agreement of the MSs.

¹ Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers, *The EFSA Journal* (2007), 96, 1-46.

The information to be reported by MSs is outlined in subsections 7.2 to 7.8, and consists of two broad categories: description of the programme (7.2 to 7.5); and individual data for each sample (7.6 to 7.8).

The description of the programme should provide an overview of the sampling programme in its entirety in the MSs and the overall results obtained (7.2).

These descriptions will be submitted once by each MS, and should take the form of a textual account of the sampling planned, the sampling actually realised, and the results obtained. Sections 7.2, to 7.5 provide some headings to indicate the type of information to be submitted in this context.

Individual data should be submitted for each sample tested as part of the sampling programme. This information shall be submitted in a form of raw data using a 'Data Dictionary' and data collection forms established and provided by the Commission. Some information such as details of analysis and results obtained, will be required for all samples (7.6). There will also be specific information required depending whether the sample was collected from a breeding holding (7.7), or from a production holding (7.8). Sections 7.6 to 7.8 will be converted into a 'Data Dictionary' format to facilitate reporting of these data.

7.2. Overview of the sampling programme and results

- Member State name
- Date of beginning and finishing the sampling and analysis
- Number of samples obtained and analysed:
 - From breeding holdings
 - From production holdings
 - From holdings sampled for within-holding prevalence study
- Overall results:
 - Prevalence of breeding holdings and of production holdings infected with *Salmonella*, and serovars of *Salmonella*
 - Outcome of the within-holding prevalence study

7.3. Overview of the breeding holding survey results

- Description of the population of breeding holdings in a Member State:
 - Total number of breeding holdings
 - Total number of nucleus holdings
 - Total number of multiplier holdings
 - Approach to use *Salmonella* vaccination in breeder holdings
- Number of breeding holdings planned to be sampled, and number of breeding holdings actually sampled
- Comment on overall representativeness of the breeding holdings sampling programme

7.4. Overview of the production holding survey results

- Description of the population of production holdings in a Member State:
 - Total number of production holdings
 - Total number of farrow to weaner holdings
 - Total number of farrow to grower holdings
 - Total number of farrow to finish holdings
 - Approach to use *Salmonella* vaccination in production holdings
- Number of production holdings planned to be sampled, and number of production holdings actually sampled
- Comment on overall representativeness of the production holdings sampling programme

7.5. Overview of the laboratory analysis

- For each laboratory involved in *Salmonella* analysis:
 - Laboratory identifier code
 - NRL for this organism

7.6. Holding-level variables for all samples

- Code of the holding
- Holding production type:
 - Indoor versus ‘any stage of the production kept outdoors’
 - Nucleus, multiplier, farrow to weaner, farrow to finish, and farrow to grower
- Holding size: the number of breeding pigs present at the time of sampling (adult inventory)
- Replacement policy: all replacement breeding pigs purchased; some replacement breeding pigs homebred, or all replacement breeding pigs homebred
- Clinical symptoms of diarrhoea: Were there symptoms of diarrhoea within the 3 months before the sampling?
- Use of *Salmonella* vaccination

7.7. Sample-level variables for all samples

- Code of the laboratory involved in initial analysis
- Date of sample collection
- Date laboratory analysis begun
- Detection of *Salmonella*:
 - Qualitative result (positive/negative)
- Serotyping of *Salmonella*:
 - Serovar(s) detected (may be more than one)

- Age of the pigs: all gilts versus mixed age breeding pigs
- Sex: only sows; sows and boars or only boars
- Production stage: maternity; mating, gestation, other
- Housing: slatted floor (fully/partly); solid floor; deep straw or other
- Diet: are pigs in this pen, yard, or group fed compound feed exclusively or not?
- Feed supplement: is there any substance able to control *Salmonella* added to the feed (such as organic acid or a probiotic)?
- Use of antimicrobials: are antimicrobials systematically used in all animals of this group?
- The last date of administration of antimicrobials to the animals (within the last four weeks)?

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Annex I. Rationale for secondary sample size calculation (within-holding number of samples), and for pooling and estimation of herd-level *Salmonella* status¹

1. Introduction

The purpose of the survey is to classify each selected holding with respect to *Salmonella* infection as positive or negative. The prevalence of infected animals within the holding is not to be estimated. Pooling of faecal samples offers a simple method of reducing the number of laboratory samples to be tested and also provides for simple sample collection methods in the field.

Collection of freshly voided faecal material represents the most feasible means of sampling. Use of rectal swabs is disappointing and not recommended. If the minimum within-holding prevalence to be detected is low (<10%), as is likely to be the case, then there will be efficiency gains to be made from pooling.

However, there are an important series of assumptions that underlie the use of pooled samples and these are discussed below.

2. Detection of infection within a pen of pigs – comparing individual to pooled samples

The likelihood of detecting *Salmonella* from an individual sample from one randomly selected pig in one pen will depend upon:

- Probability that the pig is infected (the within-pen prevalence)
- Probability that the pig is excreting *Salmonella*, given that it is infected
- Sample weight
- Probability that the sample will contain sufficient bacteria to be detected (including the probability that the sample does not contain any inhibitory substance)
- Probability that the isolation process will yield a growth of *Salmonella*, given that it is present

Traditional approaches could be used to decide how many pigs from a pen should be tested to be sure of detecting at least one positive pig, given the within-pen prevalence, the number of pigs within a pen and the desired level of certainty. Figure 1 below indicates the number of individual samples that would be required, according to herd size.

¹ Prepared by Alex Cook and Mark Arnold, CERA, VLA-Weybridge.

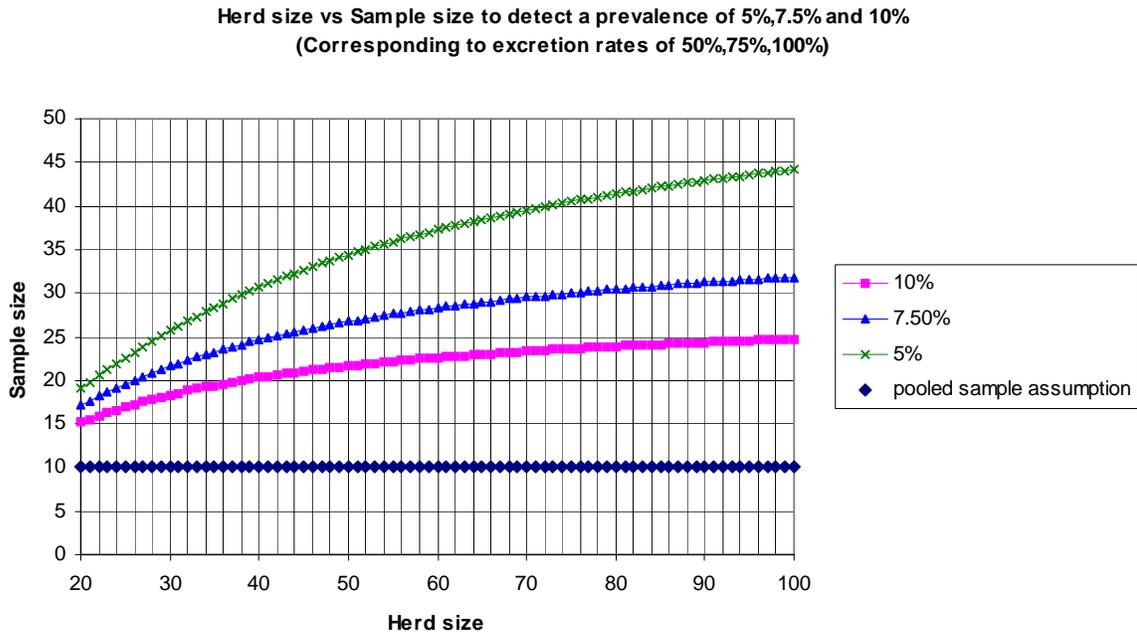


Figure 1. Within-herd sample size according to herd size

However, in practice it would be more challenging to collect an individual sample from the pig per rectum and simpler to choose to collect freshly-voided faeces. Thus, actually the probability of sampling a positive faecal sample is estimated and the assumptions must be extended to assume that infected pigs shed the same number of faecal pats as uninfected pigs. An alternative choice would be to collect a pooled sample from a pen. Then, further assumptions must be taken into account:

- Probability that at least one infected and excreting pig contributes to the pooled sample
- Probability that inhibitory substances excreted by any pig are added to the pool
- Impact of a varying weight of faeces from each pig that contributes to a pool.

Previous work demonstrated that pooled pen sensitivity was greatest when the greatest number of pigs within a pen were included within the pooled sample, which was fixed at a total weight of 25g (Arnold et al 2005). In the UK this led to the use of an approach for sample collection in finishing pigs that comprises drawing a large swab in a zigzag pattern through the pooled faecal material in the dunging area of a pen. The sensitivity of the pooled sample, allowing for the weight of faeces contributed by any individual pig to vary, but assuming that 90% of pigs contribute to less than 1g and no pig contributes to more than 10g to a 25g sample, increases as the within pen prevalence increases.

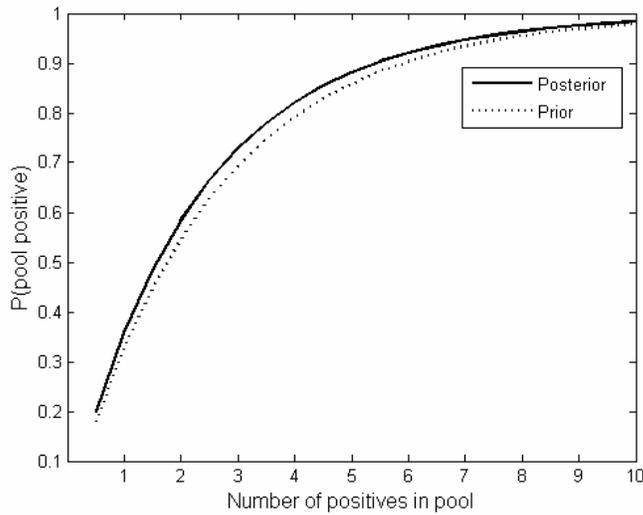


Figure 2. A comparison of the prior and posterior values of the test sensitivity of a pooled pen faecal sample between 1 and 10 true-positive samples in a pool of 10.

3. Variation between pens

Analysis of UK data showed that the prevalence of infection within pens and the prevalence of infected pens varied greatly. Furthermore, there was an important cluster effect, so that the variation in prevalence amongst pens on one holding was different to that observed on other holdings. Figure 3 demonstrates that consideration of this cluster effect slightly reduces the probability of a pooled sample testing positive at an assumed prevalence of 0%-50% of infected pigs in the herd but that the probability of any single pooled sample testing positive remains much greater than the probability of any single individual sample testing positive.

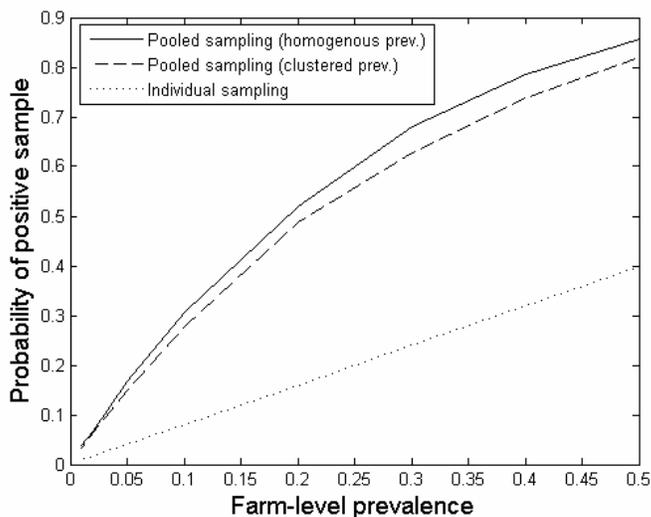


Figure 3. Probability of a pooled-pen sample testing positive, (i) where each pen has equal prevalence (homogenous prevalence), (ii) where pen-prevalence follows a gamma distribution

with a holding-level mean (clustered prevalence) and, (iii) individual-level sampling, assuming 25g of faeces and a population size much larger than the number of samples taken.

Using the assumptions described above, albeit was possible to estimate the number of pooled samples that should be collected to detect at least one positive sample over a range of assumed prevalence of *Salmonella* infection (Table 1).

Table 1. Estimated number of pooled samples to be collected to detect at least one positive sample with 80, 90 and 95% certainty over a range of *Salmonella* prevalence.

Certainty of detection	Percentage of infected pigs in population							
	50	40	30	20	10	5	2	1
95	2	3	3	5	10	19	45	91
90	2	2	3	4	7	15	37	73
80	1	2	2	3	5	10	25	48

As shown in this table, the required number of pooled samples increases as the prevalence of infection reduces. As expected, the number of samples required also increases as the certainty of detection increases. Thus, for example, if 10 pooled samples were collected per holding, it would be 95% certain of detecting at least one positive sample if the true prevalence were 10% and 80% certain of detecting at least one positive sample if the true prevalence were 5%. These results are based on Arnold et al 2005 and on further data derived from an additional 8 UK holdings. These latter results are currently being prepared for publication and are expected to submit to a peer-reviewed journal by October 2007.

4. Discussion and recommendations

On the basis of the above, it is recommended that 10 pooled samples are collected at each selected holding. These samples should be taken from randomly selected groups, pens, or yards of pigs, where at least 10 individual pigs contribute to each pool. Each sample should total 25g, and two approaches may be employed to collect these:

1. Where there is an accumulation of mixed faeces within an area of a pen or yard, then a large swab can be used to pass through the faecal mass, ensuring that at least 25g of mixed material is collected
2. Where there is no such accumulation, for example in a large yard, in a farrowing house, or other accommodation with low numbers of pigs per group, individual pinches should be selected from individual fresh faecal masses so that a minimum of 10 individuals contribute to a total sample volume of at least 25g.

Approach 1 is to be preferred, where practical. Note that this approach relies on random selection of the pens or groups to be sampled. It is appreciated that there may be particular areas on a holding where the probability of isolation of *Salmonella* may be higher, e.g. boar pens, pens with recently mixed sows etc. If such pens were selected at random, then they should be sampled, but not otherwise. The assumed cut-off of 10% overall breeding herd prevalence to classify a herd as positive or negative can only be tested using the random sampling approach. Where, on small

holdings, or holdings with large numbers of pigs kept outdoors in paddocks, the sample size is greater than the number of pens or groups, then repeat sampling of the same pen or group is required, so that a total of 10 pooled samples are submitted. Note that even in these smaller herds, the total number of individual samples that would be required is greater than the 10 pooled samples that are requested (see figure above).

5. References

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- Cowling, D.W., Gardner, I.A., Johnson, W.O. (1999). Comparison of methods for estimation of individual-level prevalence based on pooled samples. *Prev Vet Med* (39), 211-225.
- Evers, E.G., Nauta, M.J. (2001). Estimation of animal-level prevalence from pooled samples in animal production. *Prev Vet Med* (49), 175-190.
- Hill, A., Snary, E., Arnold, M., Cook, A. (2004). A Stochastic Transmission Model for *Salmonella* within a Grower-Finisher Pig Farm. Section 4. Report on project FZ2014.

Annex II. Details on the within-holding swab-sampling procedures

Ten pooled samples of freshly voided faeces must be taken from each holding. The samples must be collected using large swabs or gauzes (surgical dressings of loosely woven cotton) that can be used to pass through the faecal mass. It is best to prepare the sampling material beforehand (jars containing swabs or gauzes). The sampling procedure is as follows;

1. At the pen side put on two pairs of gloves on top of each other. Change the second pair for a new pair of gloves for each sample.
2. Find a safe clean place to rest the box of jars – it may be helpful to carry a stool for this.
3. Tear off the adhesive label and stick it firmly on the side of the sample jar.
4. Enter the pen or yard taking care not to tread on the area that is to be sampled.
5. Unscrew the jar lid, remove the swab and pass the swab through the top 2 inches of the pooled faeces in the main dunging areas of the pen or yard, swabbing over a 2 metre zigzag path so that all sides of the swab except for the point where the swab is held are well coated with faeces.
6. Carefully return the swab (or gauze) to the labelled sample jar so that the outside of the jar remains as clean as possible, and replace the lid securely. Avoid cross contamination.
7. Replace the jar in the box. Remove gloves and discard. Proceed to next sample site and follow instructions 1-7.
8. When all samples have been taken, seal each tray of jars inside two polythene bags.