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Guidelines for the determination of the prevalence of Salmonella contamination in consumer poultry at retail level

J.H. Spoorenberg[1], A.M. Henken[1], K. Frankena[1], S.H. W. Notermans[2], and A.W. van de Giessen[2]

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[1] Department of Animal Husbandry, Division of Animal Health and Epidemiology, Agricultural University, P.O. Box 338, 6700 AH WAGENINGEN, The Netherlands

[2] National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA, Bilthoven, The Netherlands, tel +31(0)30 2749111, fax +31(0)30 274 2971

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Rijksinstituut voor Volksgezondheid en Milieu, Postbus 1, 3720 BA Bilthoven,
tel. 030-2749111, fax 030-2742971

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Summary

A monitoring system is described to determine the prevalence of *Salmonella* in poultry meat products at retail level. For the Dutch situation chicken meat samples should be collected in 385 shops (confidence level of 95%, accepted error of 5% at an estimated prevalence of 50%). The main retail points for poultry meat in The Netherlands are real butchers, regular poulterers, market poulterers and supermarkets. They account for 95% of the chicken sold to Dutch consumers. Based on the market shares of these retailers, 65 real butchers, 44 regular poulterers, 37 market poulterers and 243 supermarkets should be sampled.

For sampling it is necessary to divide the chicken meat into four product groups: whole carcasses, parts of leg, parts of breast and other parts. Each of these groups should be sampled in the shops. So there will be 16 (4 shop types * 4 product groups) strata for which an estimate of the prevalence is determined. The strata should be sampled by collecting 740 gr. meat per product group per shop and is based on the quantity of chicken a consumer buys at a time. In total about 1550 samples have to be collected.

To compare results it will be of importance to examine the samples in an identical way. For example by rinsing the meat samples with buffered pepton water and testing the fluid for the presence of *Salmonella* using the ISO 6579 isolation method.

Based on the prevalences of the 16 strata, the prevalences for each shop type, product group and the total chicken sold can be calculated. The formula's for calculating these prevalences and the standard deviation are presented.

Although, the system is described for the Dutch situation, it can also be applied in other countries where similar data are available.

Samenvatting

Een monitoringsysteem is beschreven om de prevalentie te bepalen van *Salmonella* in pluimveevleesprodukten. Voor de Nederlandse situatie dienen pluimveevleesprodukten te worden betrokken van 385 winkels (betrouwbaarheidsniveau 95%, fout 5% en een geschatte prevalentie van 50%). Voor de Nederlandse situatie zijn 4 soorten verkooppunten te onderscheiden, te weten slagers, poeliers, straatmarkten en supermarkten. Voor de bemonstering is het verder noodzakelijk om pluimveeprodukten in 4 groepen te verdelen, te weten, hele karkassen, kippepoten, borststukken en overige delen. Van ieder van deze groepen dienen monsters te worden genomen. In totaal zijn er 16 verschillende strata (4 typen verkooppunten x 4 produktgroepen) waarbinnen een bepaling wordt uitgevoerd van de prevalentie. In elk van de strata dient 740 gram vlees te worden bemonsterd, hetgeen overeenkomt met een hoeveelheid die door de consument gemiddeld in één keer wordt gekocht. In totaal zullen aldus 1.550 monsters moeten worden verzameld.

Om de resultaten te vergelijken is het noodzakelijk dat monsters op identieke wijze worden onderzocht, bijvoorbeeld door ze te schudden met gebufferd peptonwater en de schudvloeistof te onderzoeken op *Salmonella*, met behulp van de ISO methode 6579.

Gebaseerd op de prevalentie van *Salmonella* in elk van de 16 strata kan de prevalentie voor ieder verkooppunt, produktgroep en het totale bestand worden berekend. De formules om deze prevalenties te berekenen, alsmede hun standaard deviaties, worden weergegeven in dit rapport.

Ofschoon het systeem voor de Nederlandse situatie is uitgewerkt, kan het ook in andere landen worden toegepast, mits identieke gegevens ter beschikking staan.

1 Introduction

Salmonella is a major cause of human gastro-enteritis. To decrease the number of these infections, the EU has laid down in the Council Directive 92/117/EEC that data have to be collected about prevalences of the organism in farm animal populations. Also control measures to reduce the presence of *Salmonella* in the poultry breeding population have to be taken. The Directive aims to reduce foodborne diseases by reducing infections in animal populations. At the very moment, control measures only concern elimination of *S. enteritidis* and *S. typhimurium* in poultry breeding flocks, but may be enlarged to all types of *Salmonella* in broilers.

Under ideal circumstances it can be expected that if the prevalence of *Salmonella* spp. in broiler stocks is reduced, this reduction can also be measured in the product (meat) at consumer level. Therefore, the success of reduction of *Salmonella* in broiler flocks can easily be measured by testing either poultry broiler flocks or by testing meat products. However, research has shown that, during slaughter, contamination from *Salmonella* contaminated carcasses to other carcasses can occur (Notermans et al., 1975). This means that meat from a flock free of *Salmonella* can become infected during processing. Also, cross-contamination can occur in the shop where the meat is sold (Smit and Nooder, 1980). This is why a monitoring system at consumer level is more appropriate to estimate more precisely human exposure. This system can also be used to determine whether or not control measures are effective.

In order to give a reliable estimate of the prevalence of *Salmonella* in poultry, several steps have to be taken. These steps have been described by Mourits et al. (1995). Essentially, they are identical for the monitoring system described in this paper which is designed to monitor the exposure of the consumer to poultry meat contaminated with *Salmonella.*

In The Netherlands the consumption of chicken accounts for over 90% of the poultry meat consumed (PVE, pers. comm.). Therefore, the steps described by Mourits et al.(1995) have been applied to chicken. This is done for the Dutch situation, but it is possible to replace the data by data from other countries. If the same system is used in these other countries, data concerning the prevalence of *Salmonella* in chicken can be truly compared.

2 Sampling procedure

This chapter describes the steps that have to be taken and the data needed to make an estimation of the prevalence of *Salmonella* in chicken meat that can be bought by the consumer.

2.1 Selection of sampling population

2.1.1 Population to be investigated

The first step in the survey is to determine the population that has to be sampled. This is the amount of chicken meat that is bought by the Dutch consumer. Since it is hard to define a population based on meat, one may start with the population of retail points. This population is the total number of retail points (shops) where the consumer can buy chicken. Table 1 shows the market shares for chicken of several distribution channels.

Table 1. Market shares for chicken of several distribution channels in 1994 (PVE (1995) and EIM/CRR (1995))

Type of channel	Market share in chicken (in %)	Market share in consumer chicken (in %)	Market share in consumer chicken excluding Other (in %)
Real butcher	12	16	17
Regular poulterer	7	10	11
Market poulterer	6.4	9	9
Supermarket	46	60	63
Non-consumer channel[1]	25		
Other	3.6	5	

[1] Restaurants, institutions etc.

From Table 1 can be concluded that there are several channels for chicken distribution. Through the non-consumer channel no chicken is purchased by consumers, so this channel is

excluded. This leads to the market shares for consumer chicken. Because it is hard to determine the retail points in the "Other" channel (farmsales, delicatessen shops, etc.), this channel is excluded as well. So, there are 4 retail channels which will be included in the survey. They comprise:

1) **real butchers**: they sell fresh meat and pre-packed (fresh) meat

2) **regular poulterers**: they sell products from a shop

3) **market poulterers**: they sell products on (different) street markets

4) **supermarkets**: there are several ways for supermarkets to present chicken to their consumers;

- through a private butcher: sells fresh meat and prepacked (fresh) meat
- through a "chain" butcher: sells fresh meat and pre-packed (fresh) meat
- (fresh) pre-packed meat (no butcher present)

As a result the following types and numbers of shops can be indicated as the population to be sampled (Table 2).

Table 2. Number of shops in the sampling population (Source:EIM/CRR (1995) and CBL (pers. comm.))

Shop type	Number	Year
real butchers	4655	1995
regular poulterers	579	1995
market poulterers	200	1995
supermarkets[1]	7274	1994
total	12708	

[1] The exact number of supermarkets selling fresh (chicken) meat is not known. The number of supermarkets presented here is the total number of supermarkets. Of this number a (very) small percentage does not sell meat. Also the distribution of the several types is not exactly known. Therefore, no distinction will be made between the different types.

Table 2 shows that there are 12708 retail points in the population from which samples have to be taken and to be examined for the presence of *Salmonella*. To examine all these retail points would be to expensive and time consuming. Therefore, a smaller number will be selected as a

random sample. Meat from these retail points will be examined for the presence of *Salmonella*. The size of this sample will be described in the next paragraph.

2.1.2 Primary sample size

To calculate the number of retail points to be sampled Formula 1 can be used. This number is called the primary sample size n:

$$n = \left(\frac{Z_{\alpha} * SD}{L} \right)^2 \quad (1)$$

Where:

SD = standard deviation $[P(1-P)]^{1/2}$ (2)

P = the expected prevalence

L = the accepted error. This resembles the accepted error in the estimation of the prevalence. When L equals 5%, the estimated prevalence is allowed to vary 5% (e.g. 50 ± 5%).

Z_{α} = the value for normally distributed data at specified confidence level α (see Table 4), where α resembles the chance that the real prevalence lies between the estimated prevalence ± the accepted error (e.g. 95% chance that the true prevalence is between 50 ± 5%).

From Formula 1 and 2 it can be concluded that in order to determine the primary sample size a "guestimate" of the prevalence of *Salmonella* is needed. This prevalence is used to calculate the SD. The SD of a proportion solely depends on that proportion and can be calculated with Formula 2. From Table 3 it appears that SD is maximal when the expected prevalence equals 50%.

Table 3. Relationship between Prevalence and Standard Deviation (SD)

Prevalence	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**	**0.60**	**0.70**	**0.80**	**0.90**
SD	0.30	0.40	0.46	0.49	0.50	0.49	0.46	0.40	0.30

The highest estimated prevalence of *Salmonella* in chicken products is 48% (Van der Zee and De Boer, 1995). The other parameters, L and α, are determined by the investigator.

Table 4. Confidence levels (α) and corresponding Z_α-values (2-sided)

α (%)	80	90	95	99
Z_α	1.28	1.64	1.96	2.58

If the primary sample size exceeds 5% of the total population (N), n has to be adjusted with Formula 3:

$$n_{adj} = \frac{n}{\left(1+\frac{n}{N}\right)} \tag{3}$$

EXAMPLE. *In a group of 100 real butchers it is expected that 10 per cent of the shops are contaminated with* Salmonella *spp. To confirm this, a number of real butchers will be investigated for the presence of the contamination. The accepted error is set at 5% and the confidence level at 95% (α=0.95 --> Z_α =1.96). Then:*

$$n = \left(\frac{Z_\alpha * SD}{L}\right)^2 = \left(\frac{1.96 * 0.30}{0.05}\right)^2 \approx 139$$

Note that n essentially is independent of N and, thus, indeed can be larger than N! If so, surely, finite population correction must take place.

*Use of these data results in a sample size of 139 shops but since the sampling fraction is larger then 0.05*N (and larger than N), the sample size can be reduced to 59. This means that by sampling 59 shops it can be demonstrated that with a probability of 95% the prevalence of the contamination is between 5% and 15%.*

2.1.3 Stratification

Stratification means that the population is divided into groups. This division is made to produce a gain in precision in the estimate of the prevalence. The groups are based on similarities between the sample units. In this way sampling units can be divided in uniform groups. This leads to a reduction in deviation within groups. Thus, the prevalence for each stratum can be calculated with a greater reliability. In this survey 4 strata will be defined. They are the 4 retail channels indicated in paragraph 2.1.1.:

1 **real butchers**

2 **regular poulterers**

3 **market poulterers**

4 **supermarkets**

The definition of these strata is based on type of shop. It also deals with differences that may occur because of differences in suppliers. It is very likely that a chain of supermarkets uses one or two big poultry processors to supply all their stores, whereas a butcher or poulterer purchases chicken from a smaller, local processor.

Another difference between supermarkets and butchers/poulterers is the fact that in supermarkets almost all meat is sold pre-packed from a self service shelf. In the other shops most of the meat is sold over the counter.

The different types of supermarkets are taken together. This is done because of lack of information about the market shares of each type. When, in the future, this information becomes available, more strata can be defined.

Because we are interested in the risk for a consumer to buy *Salmonella* contaminated meat, the number of selected retail points in each stratum will be based on the market share of that stratum (Table 1). So, 63% of the retail points to be sampled will be supermarkets, 17% butchers, 11% regular poulterers and 9% market poulterers. This is called proportional allocation based on stratum weight.

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This is not always the correct procedure, but because the estimated prevalences (and therefore the deviation) and the cost of sampling are the same for each stratum it is the correct way. If sampling shows that the prevalences or the costs of sampling per stratum are not the same in each stratum a correction must be made. For this correction a formula is given by Mourits et al. (1995). This correction assures that the sampling is done in the most efficient way.

To make sure that no region in the country will be under- or overrepresented in the prevalence estimation each stratum will be divided for region (Table 5). For butchers in supermarkets and real butchers the number of shops in a province corresponds well with the population of the province: regarding poulterers this corresponds less (EIM/CRR, 1995).

Table 5. Distribution of retail shops over the provinces in 1995 (Bedrijfschap Slagersbedrijf (pers. comm.) and EIM/CRR (1995))

Province	Real butchers		Butchers in supermarkets		Regular poulterers and market poulterers	
	#	%	#	%	#	%
Groningen	168	3.6	109	4.2	16	2
Friesland	213	4.6	127	4.9	21	2.7
Drenthe	114	2.4	102	3.9	16	2
Overijssel	302	6.5	181	7.0	50	6.4
Flevoland	31	0.7	48	1.9	9	1.2
Gelderland	524	11.3	342	13.2	140	18
Utrecht	273	5.9	165	6.4	60	7.7
Noord Holland	826	17.7	405	15.7	117	15
Zuid Holland	1004	21.6	437	16.9	195	25
Zeeland	139	3.0	66	2.6	10	1.3
Noord Brabant	646	13.9	419	16.2	86	11
Limburg	415	8.9	183	7.1	60	7.7
Total	4655	100	2584	100	780	100

Note that no distinction is made between market poulterers and regular poulterers. This is because no data are available. It is likely that the number of market poulterers in a province is about 26% of the total number of poulterers in that province (see Table 2).

2.2 Estimating the prevalence in the population

After the shops to be sampled have been determined, the prevalence of *Salmonella* contaminated chicken can be estimated. This means that each selected shop has to be examined for the presence of *Salmonella* spp. in chicken. Samples of meat have to be taken and these will be tested for the presence of *Salmonella*. This is called secondary sampling.

2.2.1 Secondary sample

The aim of this survey is to determine the exposure of the consumer to *Salmonella* contaminated chicken. In other words, what is the probability that the consumer buys contaminated meat. Ideally it would be best to determine the prevalence of *Salmonella* contaminated meat in each shop. This would be similar to what is done on a farm where a number of animals is examined for infection. An animal is the unit to be examined. With a certain number of examined units it is possible to give a reliable estimate of the prevalence (see 2.1.2). In a shop, however, there are no clear units of meat that can be examined. This means that another solution has to be found. Since we are interested in the risk for a consumer to buy *Salmonella* contaminated meat, the best sample unit is what a consumer buys at a time. Then, based on the results of the examination of these units, the prevalence can be calculated per group of shops. This approach means that it has to be known which products the consumer buys and how much is bought at a time.

2.2.2 Products

There is a wide range of chicken products that can be purchased in a shop. These products include several types of fresh meat (whole carcasses, wings, fillets, organs etc.), fresh frozen meat (whole carcasses, wings, fillets, organs etc.), fresh processed meat (with spices and herbs added) and meat products (cold cuts, sausages etc.). The last two categories will be excluded

from determination for *Salmonella*. It is known that paprika powder may be a source of *Salmonella* (Kovacs-Domjan, 1988). So, only fresh and fresh frozen meat will be included.

From research conducted by Van der Zee and De Boer (1995) it can be concluded that the different kinds of fresh meat are not equally contaminated with *Salmonella*. Therefore, it seems best to estimate the prevalence of *Salmonella* contamination per product or product group. This means that more than one prevalence estimation must be made.

All these estimations together, combined with market shares can be used to calculate the overall prevalence of *Salmonella* in chicken bought by consumers.

The following data on market shares are available. Ninety eight percent of the chicken consumed is broiler chicken, the other 2 percent is soup chicken (PVE, pers. comm.). In 1993, 8.7% of chicken sold in supermarkets, was sold frozen (CBL, pers. comm.). The consumption can be divided into 4 different groups (Table 6). The consumption of soup chicken is included in these four groups. No distinction is made between fresh and fresh frozen meat. The market share of each of these groups is known for the 4 types of shop that were defined in paragraph 2.1.1.

Table 6. Market shares of chicken for each product groups per type of shop in 1994 (PVE,pers. comm.)

Shop type	**Product group**			
	whole carcasses	**parts of leg**	**parts of breast**	**other parts**
real butcher	3.1	27.3	61.6	8.0
regular poulterer	14.4	31.1	30.4	24.1
market poulterer	13.1	28.8	33.3	24.9
supermarket	11.1	30.5	35.9	22.5

From these four groups, representative samples have to be taken. As a result, 4 samples, one for each product group, have to be collected in every selected shop.

The choice to split the meat into different groups is in a way a further stratification of the population. This means that, instead of 4 strata, 4x4 strata are defined.

2.2.3 Consumption

The size of the sample taken in the shop should have the same size as the portion of meat that the average consumer buys at a time. In this way the sample will be the most representative. In Table 7 the average amount of chicken bought per visit is shown.

Table 7. Amount of chicken (kg) bought per purchase per product group and shop type in 1994 (PVE, pers. comm.)

Shop type	Product group				
	total chicken	whole carcasses	parts of leg	parts of breast	other parts
real butcher	0.77	1.24	1.03	0.66	0.52
regular poulterer[1]	1.08	1.17	1.17	0.74	0.82
market poulterer[1]	1.08	1.17	1.17	0.74	0.82
supermarket	0.64	1.28	1.02	0.50	0.41

[1] no separate data were available for the types of poulterers

The average purchase over all product groups and shop types amounts to 0.73 kg.

2.3 Detection of *Salmonella*

After the samples have been taken and transported to the laboratory under conditions that neither allow multiplication of the organism or injury they are analyzed for the presence of *Salmonella*. It is evident that identical sample preparation and isolation procedures have to be applied in order to compare results.

An appropriate method of sample preparation consists of placing the sample (with a minimum weight of 250 gram) into a plastic bag and to add an identical quantity of buffered peptone water to the test sample. Following, the plastic bag with its contents is shaken on a rotary shaker at 100 rpm for 5 min. Thereafter, 250 ml of the peptone water is put into a glass jar.

For isolation of *Salmonella* from the buffered peptone water the ISO 6579 method is recommended. For this, the jar with buffered peptone water is incubated at 37 °C for 18 hours. Thereafter, 0.1 ml is transferred into a tube containing 10 ml RV-medium and incubated at 43 °C for 48 hours. After 24 and 48 hours of incubation a loopfull fluid is streaked onto Brilliant Green Agar plates. After incubation of these plates at 37 °C for 18 hours, characteristic colonies are biotyped and serotyped.

The sensitivity of the method described above can not be predicted but is not 100%. Therefore, the use of reference material is obligatory. Due to the bio- and serotyping of the isolates the specificity of the test is 100%

2.4 Calculation of prevalences

2.4.1 Prevalence per stratum

The prevalence of *Salmonella* contamination in product group i in each stratum can now be calculated. This will result in a matrix of prevalences. The size of this matrix will be (4x4) = (the number of product groups x the number of shop types). So, in total there are 16 strata with an estimated prevalence.

The prevalence in a stratum is the number of positive samples in that stratum divided by the total number of samples tested in that stratum.

In formula:

$$\bar{p}_{i,a} = \frac{\#positive_{i,a}}{total_{i,a}} \qquad (4)$$

Where $\bar{p}_{i,a}$ = (estimated) prevalence of *Salmonella* contaminated product i in shop type a

The prevalence can be expressed as a proportion or as a percentage (proportion x 100). In this survey percentages will be used.

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Because the sensitivity of the test is not 100% the prevalence will be underestimated, as the following formula shows:

$$TP = \frac{AP + SP - 1}{SE + SP - 1} \quad (5)$$

where

TP	=	true prevalence
AP	=	observed (measured) prevalence
SP	=	specificity of the test
SE	=	sensitivity of the test

As stated in paragraph 2.3 the specificity of the test is 100%, the true prevalence depends on what is measured (AP, in this case $\bar{p}_{i,a}$) and the sensitivity. Since the latter is less then 100%, an underestimation of the prevalence is observed. This means that in all laboratories the same test procedure has to be used, otherwise the results are not comparable. If not so, the results should be corrected with the formula above; however this is not possible because the sensitivity is not known.

The precision of the population estimate $\bar{p}_{i,a}$ can be visualized by calculating its variance or standard deviation (SD). To calculate SD Formula 2 can be used:

$$SD = [P(1-P)/n]^{1/2} \quad (2)$$

Since only an estimate of P (prevalence) is known, SD is estimated by Formula 6:

$$sd = [\bar{p}_{i,a}(1- \bar{p}_{i,a})/n]^{1/2} \quad (6)$$

With the standard deviation it is possible to calculate a confidence interval for $\bar{p}_{i,a}$. Such a confidence interval represents the range of possible values for $\bar{p}_{i,a}$ that is consistent with the observed data. A 95% confidence interval implies that, with a probability of 95% (Z_α=1.96), the estimated $\bar{p}_{i,a}$ of a new sample lies between the limits of that interval. These limits are called the approximate 95% confidence limits for $\bar{p}_{i,a}$ and are calculated using the following formula:

$$\bar{p} - 1.96 * (\bar{p}(1-\bar{p})/n)^{1/2} \quad \text{and} \quad \bar{p} + 1.96 * (\bar{p}(1-\bar{p})/n)^{1/2} \quad (7)$$

2.4.2 Other prevalences

The prevalence per product per stratum is now known. By combining this prevalence with other data more information can be found.

a) Prevalence of *Salmonella* contaminated meat per type of shop

Based on the prevalences in the strata of one shop type and the market shares of these strata, the (weighted) prevalence of *Salmonella* contamination in chicken in that shop type can be determined.

In formula:

$$\bar{p}_a = \frac{\sum p_{i,a} * MS_{i,a}}{MS_A} \tag{8}$$

where

$\bar{p}_a$ = prevalence of *Salmonella* contaminated chicken in shop type a

$\bar{p}_{i,a}$ = prevalence of contaminated product i in shop type a

$MS_{i,a}$ = market share of product i in shop type a

MS_A = market share of shop type a in chicken sales

From this prevalence the standard deviation and confidence interval can also be estimated.

In this stratified random sample the estimate of the standard deviation is:

$$sd = \sqrt{\sum \left(\frac{MS_{i,a}}{MS_A}\right)^2 * \frac{\bar{p}_{i,a}(1-\bar{p}_{i,a})}{n_a - 1} * \left(1 - \frac{n_a}{N_a}\right)} \tag{9}$$

where:

n_a = the number of samples for shop type a

N_a = the number of shops of type a

$1-\frac{n_a}{N_a}$ = the finite population correction

The finite population correction (fpc) can be ignored whenever the sampling fraction (n/N) does not exceed 5%.

Because of reasons stated by Mourits et al. (1995) the formula underestimates the standard deviation. Therefore, if the data are to be compared over different sampling periods, the maximal standard deviation must be used. The standard deviation is maximal when the estimate prevalence equals 50%. The maximal SD then is

$$sd_{\max} = \sqrt{\sum\left(\frac{MS_{i,a}}{MS_A}\right)^2 * \frac{0.5*(1-0.5)}{n_a-1} * \left(1-\frac{n_a}{N_a}\right)} \qquad (10)$$

with a corresponding 95% confidence interval of

$$\bar{p}_a \pm 1.96*\mathrm{sd}_{\max}$$

b) Prevalence of *Salmonella* contaminated meat per product group

Based on the prevalences in the strata of one product group and the market shares of these strata, the prevalence of *Salmonella* contamination in chicken of that product group can be calculated.

In formula:

$$\bar{p}_i = \frac{\sum p_{i,a} * MS_{i,a}}{MS_I} \qquad (11)$$

where

$\bar{p}_i$ = average prevalence of *Salmonella* contaminated meat in product group i

MS_I = market share of product group i in chicken consumption

The standard deviation can be calculated with the following Formula:

$$sd = \sqrt{\sum \left(\frac{MS_{i,a}}{MS_I}\right)^2 * \frac{\bar{p}_{i,a}(1-\bar{p}_{i,a})}{n_a - 1} * \left(1 - \frac{n_a}{N_a}\right)} \qquad (12)$$

Analog to Formula 10 the maximal standard deviation can be calculated.

c) Prevalence of *Salmonella* contamination in the total meat consumption

To be able to give an estimate of the chance that a consumer purchases a *Salmonella* contaminated portion of chicken, the overall prevalence of *Salmonella* contamination has to be calculated. This prevalence is based on the prevalences of all the strata and their share in chicken consumption.

In formula:

$$\bar{p}_{total} = \frac{\sum p_{i,a} * MS_{i,a}}{\sum MS_{i,a}} \qquad (13)$$

where

$\bar{p}_{total}$ = prevalence in total meat consumption

$MS_{i,a}$ = market share of stratum i,a

To calculate the standard deviation, Formula 14 can be used

$$sd = \sqrt{\sum (MS_{i,a})^2 * \frac{\bar{p}_{i,a}(1-\bar{p}_{i,a})}{n_{i,a} - 1} * \left(1 - \frac{n_{i,a}}{N_{i,a}}\right)} \qquad (14)$$

2.5 Repetition of survey

The sampling procedure described above can be used if the prevalence is estimated for the first time. Once a prevalence estimation has been made, this estimate can be used to optimize the sampling procedure for the next estimate. This can result in a smaller sample size or a different allocation of the samples to the strata. Once the new primary sample size has been determined, the samples can be allocated to the different strata with the formula given by Mourits et al. (1995). This formula ensures optimal allocation based on the prevalences in the strata and the cost of sampling in a stratum. If, for instance, the prevalence in a stratum is very low, the number of samples for that stratum can be reduced.

This correction has to be made each time the survey is conducted. Also the data regarding market shares and shop numbers have to be checked for accuracy.

If the results from several surveys reveal that the prevalences of the different strata are (almost) identical, one might consider to stop with applying stratification. This depends on what one wants to monitor, it might be of interest to know the prevalence for each stratum instead of the overall prevalence.

3 Results

As indicated in the introduction of this report, a case study will be presented for the Dutch situation. This is only an example and not an indication of the real prevalence of *Salmonella* at retail level.

3.1 Selection of sampling population

As can be seen in Table 2 the number of shops in the primary population to be sampled is estimated at 12708 (N). From this population a number of shops will be selected.

3.1.1 Primary sample size

The estimated prevalence is set at 48%. This is the highest prevalence of *Salmonella* in chicken products found in surveys by Van der Zee and De Boer (1995). It is close to 50% and therefore suitable to use at all circumstances. L is set at 0.05 and $\alpha = 0.95$.
Based on the estimated prevalence, the estimated standard deviation can be calculated with Formula 2:.

$$sd = [0.48(1-0.48)]^{1/2} = 0.50$$

The primary sample size then equals (Formula 1)

$$n_{pr} = \left(\frac{1.96 * 0.50}{0.05}\right)^2 = 384.16 \approx 385$$

Since 385 is approximately 3% of the total population, adjustment with Formula 3 is not necessary.

3.1.2 Stratification

The number of shops in the primary sample size has to be allocated to the defined shop types. This division is based on the market share of each shop type. Table 8 shows the final allocation.

Table 8. Allocation of primary sample size to the shop types

Shop type	Market share (in %)	# Shops to be sampled
real butchers	17	64
regular poulterers	11	43
market poulterers	9	35
supermarkets	63	243
total	100	385

The number of shops in each group has to be distributed over the provinces. Table 9 shows the results of this distribution.

Table 9. Distribution of shops to be sampled over the provinces

Province	Real butchers		Supermarkets		Regular poulterers		Market poulterers	
	%	#	%	#	%	#	%	#
Groningen	3.6	2	4.2	10	2	1	2	1
Friesland	4.6	3	4.9	12	2.7	1	2.7	1
Drenthe	2.4	2	3.9	10	2	1	2	1
Overijssel	6.5	4	7.0	17	6.4	3	6.4	2
Flevoland	0.7	1	1.9	5	1.2	1	1.2	1
Gelderland	11.3	7	13.2	32	18	8	18	6
Utrecht	5.9	4	6.4	16	7.7	3	7.7	3
Noord Holland	17.7	11	15.7	38	15	6	15	5
Zuid Holland	21.6	14	16.9	41	25	11	25	9
Zeeland	3.0	2	2.6	6	1.3	1	1.3	1
Noord Brabant	13.9	9	16.2	39	11	5	11	4
Limburg	8.9	6	7.1	17	7.7	3	7.7	3
Total	100.1	65	100	243	100	44	100	37

From Table 9 it can be concluded that some shop types will be over represented. This is the result of rounding upwards in provinces where the number was smaller than 1. In fact all numbers should be rounded upwards, but this would cause a major increase in the number of shops to be sampled. Because the distribution over the provinces is only to make sure that no provinces are seriously under sampled, large numbers can also be rounded downwards. As a result a total of 389 shops will be sampled.

By defining different product groups a second division was made. So the sampling procedure is a 2-stage stratified sampling method. In total 16 strata are examined. Table 10 shows the number of collected samples per stratum. In every shop of the primary sample size, 4 samples will be collected, one for each product group. So in total 389*4=1556 samples of chicken have to be taken for examination.

Intermezzo----------
If, for any reason, the sample size turns out to be too large, a smaller sample size can be calculated by allowing a lower confidence level (α) or accepting a higher error level in the estimate (L). Adjusting the accepted error level is very effective. If the accepted error is raised from 0.05 to 0.10, the number of samples decreases with a factor 4. This is caused by the fact that the relation between n and L is quadratic (Formula 1).

Table 10. Number of collected samples per stratum

Shop type	Product group				Total
	whole carcasses	parts leg	parts breast	other parts	
real butcher	65	65	65	65	260
regular poulterer	44	44	44	44	176
market poulterer	37	37	37	37	148
supermarket	243	243	243	243	972
total	389	389	389	389	1556

3.1.3 Market shares per stratum

The market share of each stratum (Table 11) is determined by multiplying the market share of each shop type in chicken sales (Table 1) by the market share of each product group in that shop type (Table 6).

Shop type	Product group				Total (MS_A)
	whole carcasses	parts of leg	parts of breast	other parts	
real butcher	0.53	4.64	10.47	1.36	17.00
regular poulterer	1.58	3.42	3.34	2.65	10.99
market poulterer	1.18	2.59	3.00	2.24	9.01
supermarket	6.99	19.22	22.62	14.18	63.01
total	10.28	29.87	39.43	20.43	100.01

3.2 Prevalences

3.2.1 Prevalences per stratum

Because there are no test results available a fictive data set has been created. The prevalences per stratum are presented in Table 12.

Table 12. (Fictive) prevalences per stratum ($\bar{p}_{i,a}$) (in %)

Shop type	Product group			
	whole carcasses	parts of leg	parts of breast	other parts
real butcher	32.7	32.3	41.0	35.7
regular poulterer	33.5	32.9	40.0	36.3
market poulterer	33.0	32.5	42.0	33.5
supermarket	30.5	31.5	39.7	36.0

For these prevalences confidence intervals can be calculated with Formula 7. These calculations are not performed, because we are not primarily interested in the prevalence at stratum level.

All further calculations are based on the fictive data set in Table 12. So no conclusions about the real *Salmonella* status of the Dutch chicken meat can be obtained from these results.

3.2.2 Prevalence of *Salmonella* contaminated meat per type of shop

First, using Formula 8, the prevalence of *Salmonella* in real butcher shops is calculated:

$$\bar{p} = \frac{(32.7 * 0.53) + (32.3 * 4.64) + (41.0 * 10.47) + (35.7 * 1.36)}{17.00} = 37.94\%$$

So in this fictive population of butcher shops, 37.94% of the purchases made by an average consumer will be contaminated with *Salmonella* spp.

The standard deviation of this estimate is (Formula 9):

$$sd = \sqrt{\left(\frac{0.53}{17.00}\right)^2 * \frac{0.327(1-0.327)}{65-1} + \left(\frac{4.64}{17.00}\right)^2 * \frac{0.323(1-0.323)}{65-1} + \left(\frac{10.47}{17.00}\right)^2 * \frac{0.41(1-0.41)}{65-1} + \left(\frac{1.36}{17.00}\right)^2 * \frac{0.357(1-0.357)}{65-1}} = 0.0414 \approx 4.14\%$$

and the maximum standard deviation (as calculated with Formula 10) is 4.24%. This means that the maximal 95% confidence interval for the estimated prevalence is:

$$37.94 \pm 1.96*4.24 \rightarrow [29.63 , 46.25]$$

The prevalences, sd's and confidence intervals for the three other shop types are presented in Table 13.

Table 13. Prevalences, sd's and confidence intervals per shop type

Shop type	Estimated prevalence (in %)	sd (in %)	sd_{max} (in %)	Max. 95% confidence interval
regular poulterer	35.96	3.64	3.79	28.53 , 43.39
market poulterer	35.98	3.77	3.93	28.28 , 43.68
supermarket	35.35	1.64	1.72	31.98 , 38.72

Note that the finite population correction can be ignored for samples taken in butchershops and supermarkets. In these strata the sampling fraction does not exceed 5%. In the strata with

market and regular poulterers the sampling fractions are 17.5% and 7.4%. So finite population correction has to be applied.

3.2.3 Prevalence of *Salmonella* contaminated meat per product group

First, using Formula 11, the prevalence of *Salmonella* in whole carcasses is calculated:

$$\bar{p} = \frac{(32.7 * 0.53) + (33.5 * 1.58) + (33.0 * 1.18) + (30.5 * 6.99)}{10.10} = 31.36\%$$

So in this fictive population of whole carcasses bought in the different shop types, 31.36% of the purchases made by an average consumer will be contaminated with *Salmonella* spp.
The standard deviation of this estimate is, according to Formula 12:

$$sd = \sqrt{\left(\frac{0.53}{10.28}\right)^2 * \frac{0.327(1-0.327)}{65-1} + \left(\frac{1.58}{10.28}\right)^2 * \frac{0.335(1-0.335)}{44-1} * \left(1-\frac{44}{579}\right) + \left(\frac{1.18}{10.28}\right)^2 * \frac{0.33(1-0.33)}{37-1} * \left(1-\frac{37}{200}\right) + \left(\frac{6.99}{10.28}\right)^2 * \frac{0.305(1-0.305)}{243-1}} = 0.0244 \approx 2.44\%$$

and the maximum standard deviation (as calculated with Formula 10) is 2.63%. This means that the maximal 95% confidence interval for the estimated prevalence is

$$31.36 \pm 1.96*2.63 \rightarrow [26.21 , 36.51]$$

The prevalences, sd's and confidence intervals for the other product groups are presented in Table 14.

Table 14. Prevalences, sd's and confidence intervals per product group

Product group	Estimated prevalence (in %)	sd (in %)	sd_{max} (in %)	Max. 95% confidence interval
parts legs	31.87	2.35	2.52	26.93 , 36.81
parts breast	40.25	2.57	2.62	35.11 , 45.39
other parts	35.74	2.49	2.60	30.64 , 40.84

3.2.4 Prevalence of *Salmonella* contamination in the total meat consumption

In this fictive population the overall prevalence of *Salmonella* contamination in chicken is estimated (Formula 13) at 35.91%. This means that the average consumer who buys an average portion of chicken has a chance of 35.91% of buying *Salmonella* contaminated meat. The standard deviation of the estimate is 1.36% and the maximal 95% confidence interval is $35.91 \pm 1.96*1.40 \rightarrow [33.17 , 38.65]$.

4 Remarks and conclusions

If the sampling procedure is used to estimate the prevalence of *Salmonella* contamination during a period of time, the samples should be collected spread evenly during that period.
The number of samples to be collected depends largely on the desired precision. If the number of samples is too large, this can be reduced by accepting a lower precision. There are 2 ways to achieve this. The first and most effective way is to accept a higher error in the estimate of the prevalence. The second way is to lower the desired confidence.
It is interesting to notice that with the precision used in this survey (α=0.95 and L=0.05) the number of samples to be collected, 1556, is almost the same as the number actually collected by the Regional Inspectorates for Health Protection (which amounted to 1564 samples in 1994).

When the information described in previous chapters can be obtained, it will be possible to implement the sampling procedure in different countries.
The situation described in the previous chapters is based on prevalence estimation in chicken. If an estimation has to be made for another product, for example turkey, the same kind of data has to be collected. This means that market shares and consumption patterns have to be found for turkey. Most likely the primary sample size in that case will not differ much from the primary sample size for estimation in chicken. This is because the number of retail points will (almost) be the same. The number of samples can be much lower if it turns out that there are fewer product groups for turkey. So if the prevalence has to be estimated for turkey, also 385 shops have to be visited and sampled.
Basically this is true for most other kinds of meat. Only if a kind of meat is not very common and sold at a small number of shops, a smaller number of shops is needed.

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