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ScienceDirect
International Journal of Food Microbiology 118 (2007) 180–185
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Microbiological sampling of swine carcasses: A comparison of data obtained by swabbing with medical gauze and data collected routinely by excision at Swedish abattoirs

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Received 27 April 2007; received in revised form 28 June 2007; accepted 10 July 2007

Abstract

Swab sample data from a 13-month microbiological baseline study of swine carcasses at Swedish abattoirs were combined with excision sample data collected routinely at five abattoirs. The aim was to compare the numbers of total aerobic counts, Enterobacteriaceae, and *Escherichia coli*, recovered by swabbing four carcass sites with gauze (total area 400 cm^2) with those obtained by excision at equivalent sites (total area 20 cm^2). The results are considered in relation to the process hygiene criteria that are stated in Commission Regulation (EC) No 2073/2005. These criteria apply only to destructive sampling of total aerobic counts and Enterobacteriaceae, but alternative sampling schemes, as well as alternative indicator organisms such as *E. coli*, are allowed if equivalent guarantees of food safety can be provided. Swab sampling resulted in higher mean log numbers of total aerobic counts at four of the five abattoirs, compared with excision, and lower or equal standard deviations at all abattoirs. The percentage of swab and excision samples positive for Enterobacteriaceae at the different abattoirs ranged from 68 to 100% and 15 to 24%, respectively. Similarly, the percentages of swab samples that were positive for *E. coli* were higher than the percentages of positive excision samples (range 52 to 84% and 3 to 14%, respectively). Due to the low percentage of positive excision results, the mean log numbers of Enterobacteriaceae and *E. coli* were only compared at two and one abattoirs, respectively, using log probability regression to substitute censored observations. Higher mean log numbers of Enterobacteriaceae were recovered by swabbing compared with excision at one abattoir, whereas the numbers of Enterobacteriaceae and *E. coli* did not differ significantly between sampling methods at one abattoir. This study suggests that the same process hygiene criteria as those stipulated for excision can be used for swabbing with gauze without compromising food safety. For monitoring of low numbers of Enterobacteriaceae and *E. coli*, like those found on swine carcasses at Swedish abattoirs, the results also show that swabbing of a relatively large area is superior to excision of a smaller area.

Keywords: Slaughter swine; Process hygiene; Sampling methods; Total aerobic counts; Enterobacteriaceae; *Escherichia coli*

1. Introduction

The Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs states that food safety is primarily ensured by preventive approaches, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles (Anonymous, 2005). Microbiological criteria are useful for validation and verification of HACCP procedures and other hygiene control measures. Process hygiene criteria for mean log numbers of total aerobic counts and Enterobacteriaceae on carcasses of swine and other animals are given in the Commission Regulation. These criteria apply only to samples taken by a destructive method but the use of other sampling and testing schemes, including the use of alternative indicator organisms such as *Escherichia coli*, is allowed provided that the guarantee of food safety is at least equivalent (Anonymous, 2005).

The relative efficacy of destructive and various non-destructive sampling methods have been compared in several studies. Sampling by excision is commonly considered to be the preferred method for recovery of bacteria from beef and swine carcasses (Bolton, 2003; Capita et al., 2004), based on the

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doi:10.1016/j.ijfoodmicro.2007.07.009

assumption that higher numbers are recovered and that lower variation is achieved compared with swabbing. This is the case when excision is compared with the wet–dry technique in which swabbing is performed by using cotton-tipped stick swabs (Dorsa et al., 1996; Gill and Jones, 2000; Hutchison et al., 2005), whereas swabbing with more abrasive materials than cotton wool (e.g. polyurethane sponges or medical gauze pads) have been shown to recover bacterial numbers similar to those obtained by excision (Dorsa et al., 1996; Gill and Jones, 2000; Byrne et al., 2005; Pearce and Bolton, 2005). Sampling by swabbing is considered advantageous for the meat industry because it is less laborious than excision sampling and does not compromise meat quality. Swabbing usually covers larger carcass areas than excision and may therefore be more reliable for monitoring of *Salmonella* or other pathogenic microorganisms that occur at low numbers (Bolton, 2003).

In this paper, microbiological swab sample data from a 13-month baseline study of swine carcasses at Swedish abattoirs (Lindblad et al., in press) were combined with excision sample data collected routinely at five abattoirs during the same period. The aim was to compare the estimated numbers of total aerobic counts, Enterobacteriaceae, and *E. coli* obtained by swabbing with gauze with those obtained by excision. The results are considered in relation to the microbiological criteria in Commission Regulation (EC) No 2073/2005.

2. Materials and methods

2.1. Excision samples

Sampling of indicator bacteria on swine carcasses is performed routinely at Swedish abattoirs in accordance with the Commission Regulation (EC) No 2073/2005 (Anonymous, 2005). Sampling is normally performed once a week at alternating weekdays. During each sampling session five carcasses are sampled after dressing but before commencement of chilling. Tissue samples of 5 cm^2 each are excised from four sites: ham, back, belly and neck (total area 20 cm^2). These sampling sites are in accordance with those suggested in the EC Decision of 8 June 2001 (Anonymous, 2001), with the exception that the neck is sampled instead of the jowl. Pooled samples from each carcass are then analyzed for total aerobic counts and Enterobacteriaceae, either by laboratory personnel at the abattoirs or by commercial laboratories. In addition, analyses of *E. coli* are performed at some abattoirs.

2.2. Swab samples

A 13-month microbiological baseline study was performed from September 2004 through September 2005 (Lindblad et al., in press). Swab samples were collected from swine carcasses at the 10 largest abattoirs in Sweden by staff from the National Food Administration. The number of samples per abattoir was proportional to the annual slaughter volume and randomly distributed over the sampling period. During sampling weeks, sampling was performed on Mondays and Tuesdays by swabbing of two to nine carcasses after dressing but before commencement of chilling. As described in detail in Lindblad et al. (in press), one sterile medical gauze pad per carcass was used to swab an undelimited area of approximately 10×10 cm^2 at each of the four sites that are equivalent to those sampled by excision: ham, back, belly and neck (total area 400 cm^2). The samples were sent chilled overnight to the National Food Administration for analysis. In total, 541 swab samples were analyzed for *E. coli* and a selection of pathogenic bacteria (Lindblad et al., in press). About half of the samples were analyzed for total aerobic counts, and half were analyzed for Enterobacteriaceae.

2.3. Microbiological analyses

Excision samples were analyzed for total aerobic counts, Enterobacteriaceae and *E. coli* in accordance with methods described by the Nordic Committee on Food Analysis (NMKL). After homogenization of the samples by stomaching in peptone water, the total aerobic counts were determined by mixing 1 ml of suitable homogenate dilutions with melted plate count agar

Table 1
Occurrence and numbers of total aerobic counts on swine carcasses at different abattoirs as estimated by either excision or swab sampling

Abattoir	No. of sampling weeks	Sampling method	No. of samples	No. (%) of positive samples	Mean[a] (SD[b]) number (log CFU/cm^2)	Log A[c] (log CFU/cm^2)	No. of weeks with a mean number exceeding m[d]
A	20	Excision	105	101 (96)	3.6 (0.8)^A	4.5	5
		Swabbing	83	83 (100)	4.0 (0.4)^B	4.4	8
B	20	Excision	110	110 (100)	3.7 (0.7)^A	4.5	1
		Swabbing	57	57 (100)	3.3 (0.5)^B	3.8	0
C	9	Excision	45	42 (93)	2.7 (0.5)^A	3.4	0
		Swabbing	22	22 (100)	3.4 (0.5)^B	4.0	1
D	10	Excision	51	12 (24)	2.2 (0.9)^A	3.3	0
		Swabbing	28	28 (100)	2.9 (0.6)^B	3.6	0
E	9	Excision	45	45 (100)	3.9 (0.4)^A	4.3	3
		Swabbing	25	25 (100)	4.2 (0.3)^B	4.6	5

[a] Mean of log-transformed bacterial numbers. The results of the two sampling methods were compared for each abattoir. Means with different letters differ significantly (t-test, $P<0.05$). Results below the detection limit were estimated by using log probability regression.
[b] Standard deviation.
[c] Log arithmetic mean.
[d] Limit between satisfactory and acceptable results (4.0 log CFU/cm^2).

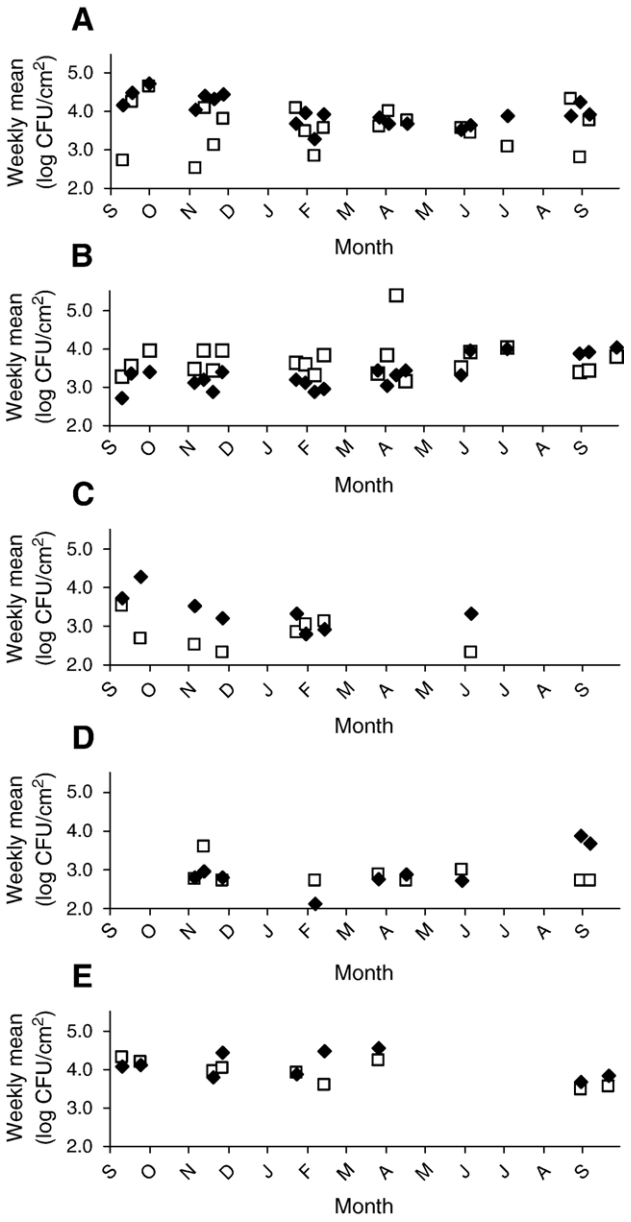

Fig. 1. Weekly mean log numbers of total aerobic counts on swine carcasses at different abattoirs (A–E) as estimated by either excision (□) or by swabbing with gauze (♦) from September 2004 to September 2005.

(PCA). The plates were incubated at 30 °C for 72 h (NMKL method 86). Enterobacteriaceae were analyzed by mixing 1 ml of homogenate or homogenate dilutions with melted violet red bile glucose agar (VRBGA). When solid, the plates were overlaid with melted VRBGA and incubated at 37 °C for 24 h (NMKL method 44). For *E. coli* enumeration, 1 ml of homogenate or homogenate dilutions was mixed with melted tryptic case soy agar (TSA) and preincubated at room temperature for 1 to 2 h. This layer was then covered with an overlay of melted violet red bile agar (VRBA), and the plates were incubated at 44 °C for 24 h (NMKL method 125).

Analyses of swab samples for total aerobic counts, Enterobacteriaceae and *E. coli* were performed according to the same NMKL methods as those used in analyses of excision samples, with minor modifications. The modifications comprised overlaying of the PCA plates with an additional layer of melted PCA and the use of VRBGA instead of VRBA in *E. coli* analyses (Lindblad et al., in press).

Table 2
Occurrence and numbers of Enterobacteriaceae on swine carcasses at different abattoirs as estimated by either excision or swab sampling

Abattoir	No. of sampling weeks	Sampling method	No. of samples	No. (%) of positive samples	Mean[a] (SD[b]) number (log CFU/cm²)	Log A[c] (log CFU/cm²)
A	19	Excision	100	16 (16)	−0.2 (1.1)^{A}	1.1
		Swabbing	87	84 (97)	0.4 (0.5)^{B}	1.0
B	15	Excision	75	18 (24)	0.3 (0.9)^{A}	1.3
		Swabbing	41	41 (100)	0.5 (0.4)^{A}	1.0
C	9	Excision	45	7 (16)	–	–
		Swabbing	22	15 (68)	−0.2 (0.9)	0.9
D	7	Excision	34	5 (15)	–	–
		Swabbing	20	16 (80)	−0.1 (0.6)	0.5

[a] Mean of log-transformed bacterial numbers. The results of the two sampling methods were compared for abattoirs A and B. Means with different letters differ significantly (*t*-test, $P<0.05$). Results below the detection limit were estimated by using log probability regression.
[b] Standard deviation.
[c] Log arithmetic mean.

2.4. Data available for comparisons between sampling methods

Excision sample data from September 2004 through September 2005 were provided by five of the largest abattoirs in Sweden (A–E) and compared with swab sample data obtained from the same abattoirs in the baseline study (Lindblad et al., in press). At three of the abattoirs (A–C) routine analyses of *E. coli* were performed in addition to analyses of total aerobic counts and Enterobacteriaceae. Only data from weeks when sampling had been performed by both excision and swabbing were included in the comparisons between sampling methods at each abattoir. Data on Enterobacteriaceae from abattoir E were excluded since the number of samples was insufficient for comparisons to be made.

Table 3
Occurrence and numbers of *E. coli* on swine carcasses at different abattoirs as estimated by either excision or swab sampling

Abattoir	No. of sampling weeks	Sampling method	No. of samples	No. (%) of positive samples	Mean[a] (SD[b]) number (log CFU/cm²)	Log A[c] (log CFU/cm²)
A	39	Excision	205	6 (3)	–	–
		Swabbing	170	94 (55)	−0.5 (0.9)	0.5
B	36	Excision	191	27 (14)	−0.2 (1.1)^{A}	1.1
		Swabbing	103	87 (84)	0.0 (0.7)^{A}	0.7
C	17	Excision	95	3 (3)	–	–
		Swabbing	44	23 (52)	−0.6 (0.6)	0.0

[a] Mean of log-transformed bacterial numbers. The results of the two sampling methods were compared for abattoir B. Means with the same letters do not differ significantly (*t*-test, $P>0.05$). Results below the detection limit were estimated by using log probability regression.
[b] Standard deviation.
[c] Log arithmetic mean.

The detection limit in analyses of Enterobacteriaceae and *E. coli* in excision samples from different abattoirs was either 0.7 log CFU/cm^2 (abattoir A, B, C, E) or 1.0 log CFU/cm^2 (abattoir D). The detection limit in analyses of total aerobic counts was higher, either 2.0 (abattoir A, B, C, E) or 2.7 log CFU/cm^2 (abattoir D), because the lowest homogenate dilutions were not routinely analyzed for this parameter. The detection limit in all swab sample analyses was −0.6 log CFU/cm^2.

2.5. Statistical analyses

Quantitative data were log-transformed (base 10) prior to statistical analyses. Based on data from either swabbing or excision, mean log bacterial numbers per carcass surface area at each abattoir were calculated. In addition, log arithmetic mean bacterial numbers (log A) were determined as suggested by Gill and Jones (2000). Log arithmetic mean numbers were calculated as:

$$\log A = \bar{x} + \ln 10 \cdot \mathrm{SD}^2/2 \quad (1)$$

where $\bar{x}$ is the mean and SD the standard deviation of log-transformed bacterial numbers (Kilsby and Pugh, 1981).

Censored results, i.e. results with numbers below the detection limit, were replaced by using log probability regression in calculations of mean log and log arithmetic mean bacterial numbers per carcass surface area at each abattoir. This method substitutes censored observations by the values predicted from a regression line fitted between the log of the non-censored observations and the corresponding quantiles of a normal distribution (le Bailly et al., 2000). Total mean log and log arithmetic mean bacterial numbers based on excision sampling were not calculated for Enterobacteriaceae and *E. coli* at abattoirs with very few (less than 10) positive samples.

Weekly mean log numbers of total aerobic counts for both sampling methods were calculated for each abattoir. Because of the limited number of samples per week, log probability regression could not be used for replacing censored results in this case. Instead, censored results were substituted by the value of the detection limit.

Since swabbing was only performed at the beginning of the week whereas excision was performed in all weekdays, excision data were examined for any bias between samples taken on Mondays and Tuesdays and on Wednesdays, Thursdays and Fridays, respectively. The mean log number of total aerobic counts from excision samples taken on the two first days or on the last three days of the week did not differ significantly (*t*-test, $P>0.05$) at any abattoir.

3. Results

3.1. Total aerobic counts

All swab samples and most of the excision samples were positive for total aerobic counts. Excision samples from abattoir D were in most cases below the detection limit (Table 1). Mean log numbers differed significantly between the two sampling methods at all abattoirs (*t*-test, $P<0.05$). Recovered mean log numbers and log arithmetic mean numbers were higher for swab samples compared with excision samples at four and three of the five abattoirs, respectively. Swabbing also resulted in equal or lower standard deviations compared with excision at all establishments (Table 1).

Only in one case, at abattoir B during the first half of the study period (Fig. 1), did excision sampling give consistently higher results than swabbing. For all other abattoirs, swabbing generally resulted in similar or higher weekly results compared with excision (Fig. 1). The limit between satisfactory and acceptable results ($m=4.0$ log CFU/cm^2) in the process hygiene criteria (Anonymous, 2005) was more frequently exceeded when the total aerobic counts were estimated by swabbing compared with excision (in total 14 and 9 of the sampling weeks, respectively) (Table 1, Fig. 1). Numbers estimated by swabbing never exceeded the limit between acceptable and unsatisfactory results ($M=5.0$ log CFU/cm^2), whereas excision sample results exceeded this limit at one occasion at abattoir B (Fig. 1).

3.2. Enterobacteriaceae

The percentages of swab samples that were positive for Enterobacteriaceae ranged from 68 to 100% at the different abattoirs. The percentages of positive excision samples were lower, ranging from 15 to 24% (Table 2). Due to the low number of positive results, mean log numbers and log arithmetic mean numbers for excision samples were only calculated for two abattoirs. At one of these, abattoir A, swabbing resulted in a significantly higher mean log number of Enterobacteriaceae compared with excision, whereas there was no significant difference between the sampling methods at abattoir B. In both cases, excision resulted in the highest log arithmetic mean numbers. Swabbing resulted in lower standard deviations compared with excision at both abattoirs (Table 2). Neither the lower limit ($m=2.0$ log CFU/cm^2) nor the upper limit ($M=3.0$ log CFU/cm^2) in the process hygiene criteria (Anonymous, 2005) were exceeded in any week (data not shown).

3.3. E. coli

The percentage of swab samples that were positive for *E. coli* ranged from 52 to 84% at the different abattoirs. The percentages of positive excision samples were lower, ranging from 3 to 14% (Table 3). The mean log number of *E. coli* from excision samples was only calculated for one abattoir (B). There was no significant difference between mean log numbers estimated from swabbing and excision at this establishment, but swabbing resulted in a lower standard deviation compared with excision. Excision resulted in the highest log arithmetic mean number (Table 3).

4. Discussion

This study shows that swabbing with gauze, as performed in the baseline study of swine carcasses at Swedish abattoirs (Lindblad et al., in press), in most cases resulted in higher mean

log numbers of total aerobic counts, Enterobacteriaceae and *E. coli*, compared with the routine excision sampling performed at the same abattoirs. Swabbing with gauze also resulted in lower standard deviations. There are two factors that may account for the relatively high bacterial numbers that were recovered by swabbing compared with excision. One is that gauze is an abrasive material with a capability of recovering bacterial numbers similar to those obtained by excision (Dorsa et al., 1996; Gill and Jones, 2000; Byrne et al., 2005; Pearce and Bolton, 2005). The second factor is that a larger area was sampled by swabbing compared with that sampled by excision, resulting in a lower variation of bacterial numbers. Bacterial numbers in food samples are assumedly log-normally distributed (Kilsby and Pugh, 1981). This has been confirmed for the distributions of total aerobic counts and Enterobacteriaceae on swine carcasses (Lindblad et al., in press). Sampling from a log normally distributed population will usually underestimate the true number and the degree of underestimation increases with increasing variance (Kilsby and Pugh, 1981). Hence, the smaller the variation that is associated with a sampling method, the higher the estimation of mean log numbers will be.

Calculating log arithmetic mean bacterial numbers resulted in somewhat different estimates of the relative efficacy of the sampling methods compared with mean log numbers. Since excision sampling resulted in higher standard deviations compared with swab sampling, the increase from mean log to log arithmetic mean numbers was larger for excision data than for swab data. As a result, the relative differences between the two sampling methods in estimating the numbers of total aerobic counts decreased at the different abattoirs, except for abattoir B where a higher mean log number was estimated by excision compared with by swabbing. Also, the highest log arithmetic mean numbers of Enterobacteriaceae and *E. coli* were estimated by excision.

Apart from resulting in more accurate estimates of average bacterial numbers, a low variation of sampling results is advantageous because it facilitates trend analyses. For instance, given a standard deviation as large as that obtained for mean log numbers of total aerobic counts determined by excision sampling at abattoir A, it would be difficult to reveal any trends in bacterial numbers. In fact, at the end of 2004, measures were taken to improve the hygienic quality of the water used in the slaughter process at this establishment. Following these improvements, at the beginning of 2005, a significant decrease in weekly mean numbers of total aerobic counts of about 0.5 log CFU/cm^2 could be estimated from swab sample results. In contrast, a significant improvement was difficult to detect by excision sampling due to the large variation in these results. At the other abattoirs, the standard deviations of the results differed less between the two sampling methods, although the variation of swab sampling results in all cases were either equal to or lower than that of excision sampling results.

The results show that it is necessary to sample a relatively large carcass area to obtain informative data on numbers of Enterobacteriaceae and *E. coli* on swine carcasses. This is especially important in order to enable trend analyses, in compliance with the requirements in the Commission Regulation (EC) No 2073/2005 (Anonymous, 2001). It is not surprising that higher percentages of carcasses were found to be positive when using swab sampling, considering that the detection limit when swabbing 400 cm^2 is about 1.3 log CFU/cm^2 lower than when excising tissue samples of 20 cm^2 (assuming equal recovery of bacteria). Gill and Jones (2000) suggested that increasing the sampling area beyond that needed to recover the bacteria of interest in more than 80% of the samples does not further enhance the determination of bacterial numbers on carcasses. Since the percentage of samples that were positive for Enterobacteriaceae ranged from about two thirds to 100% at different abattoirs, 400 cm^2 seems to be quite a sufficient area to swab at the current numbers of Enterobacteriaceae at Swedish abattoirs. For *E. coli*, the percentage of positive samples ranged from about 50 to 80%, and in this case sampling of a larger area could be considered to further increase the percentage of positive samples.

Although no process hygiene criteria for *E. coli* are stated in the Commission Regulation (Anonymous, 2001), routine sampling of *E. coli* has the advantage that this bacterium is more directly linked to fecal contamination than is Enterobacteriaceae. Also, the probability of finding pathogenic *Y. enterocolitica* on swine carcasses is related to the numbers of *E. coli*, suggesting that regular sampling of this bacterium may provide an estimate of the risk of carcass contamination with *Y. enterocolitica* (Lindblad et al., in press). The difference between the estimated mean numbers of *E. coli* and Enterobacteriaceae in the baseline study, about 0.35 log CFU/cm^2 on carcasses testing positive for both bacteria (Lindblad et al., in press), provides a possible basis for setting *E. coli* criteria in relation to the criteria stated for Enterobacteriaceae on swine carcasses in the Commission Regulation (Anonymous, 2001).

Unlike some previous evaluations of the relative efficacy of different sampling methods, where each method was applied on the same carcass site (Dorsa et al., 1996; Gill and Jones, 1998; Gill and Jones, 2000), this study was based on data obtained from sampling of different carcasses and by staff from either the abattoir or the National Food Administration. The present study has the advantage that the data used for comparisons between sampling methods originated from regular sampling over a relatively long period, either from the establishments' routine sampling programmes or from the baseline study. A disadvantage is that any differences between sampling methods may be less obvious due to the variation that was introduced by sampling of different carcasses. Also, the results may have been biased because swabbing and excision were not carried out by the same persons. Both excision and swabbing were, however, performed by more than one person at each abattoir, reducing possible systematic errors. The number of persons performing swab sampling at each abattoir ranged from two to eight, and it is noteworthy that the standard deviation of the total aerobic counts at the establishment with the highest number of persons involved in swab sampling (abattoir A) was the second lowest.

In this study, log probability regression was used for substitution of censored data in calculations of mean bacterial numbers for each abattoir. Although not previously used for estimating bacterial numbers on carcasses, this method has, for

example, been considered useful for estimating descriptive statistics of censored water quality data (Gilliom and Helsel, 1986; Helsel and Corn, 1988). Log probability regression is a robust parametric method which, compared with parametric methods such as the maximum likelihood method, has the advantage of being easy to explain and to compute (le Bailly et al., 2000).

In conclusion, the results of this study show that swabbing with gauze generally recovered bacterial numbers that were comparable with those obtained by excision, both in terms of mean log numbers per carcass surface area at different abattoirs, and the number of weeks when the limit between satisfactory and acceptable numbers of total aerobic counts were exceeded. This suggests that this non-destructive method provides guarantees of food safety that are at least equivalent to those of excision sampling, and that the same process hygiene criteria can be used for both sampling methods without compromising food safety. Considering the differences in the percentage of positive samples, the results show that swab sampling of a relatively large area is superior to excision sampling of a smaller area when monitoring Enterobacteriaceae and *E. coli* on swine carcasses at numbers as low as those found at Swedish abattoirs.

Acknowledgements

I thank the local staff at the abattoirs for performing the sampling, and the abattoir managements for sharing data from routine sampling programmes. R. Lindqvist is gratefully acknowledged for critical comments on the manuscript.

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