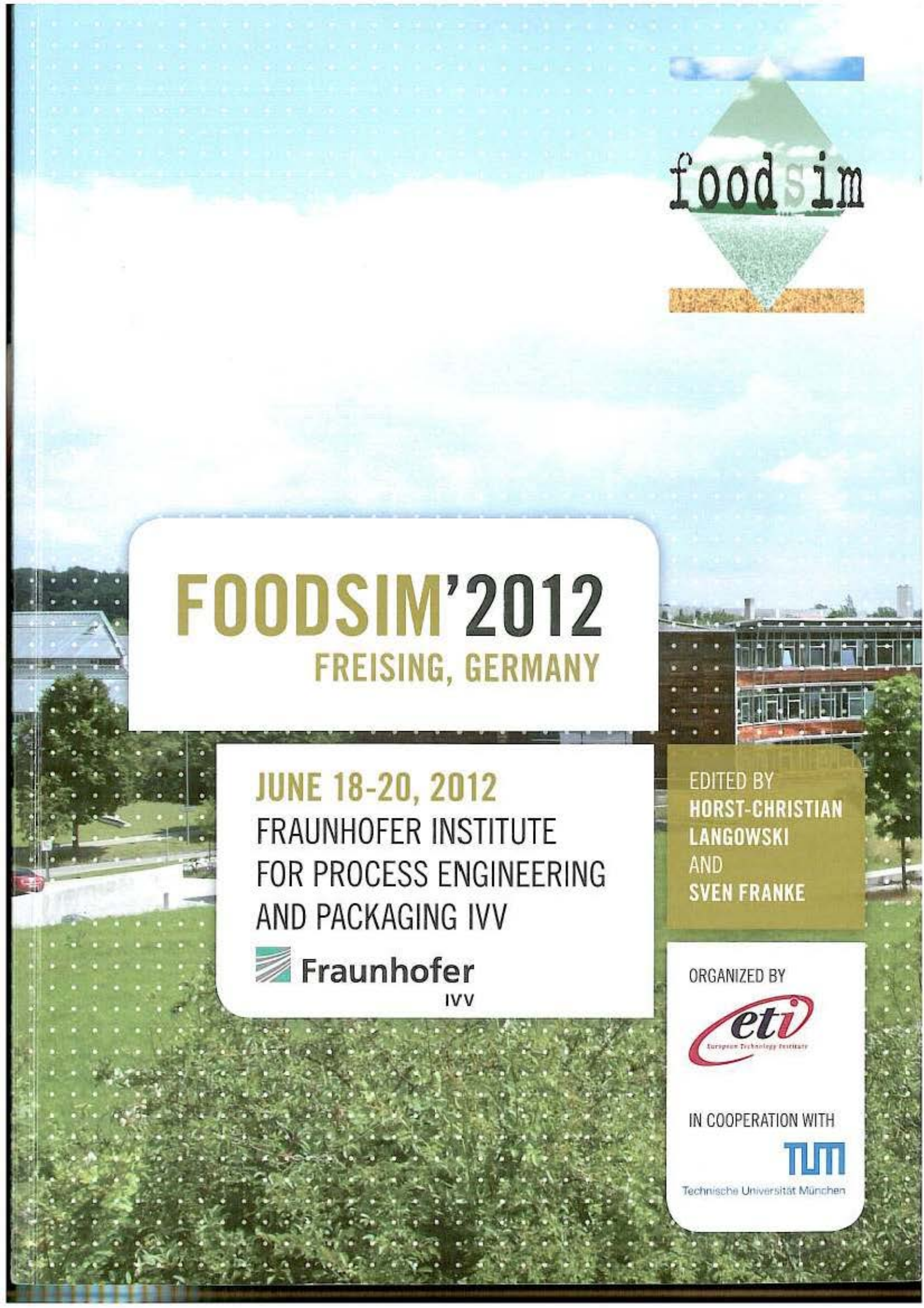

FOODSIM'2012

FREISING, GERMANY

JUNE 18-20, 2012

FRAUNHOFER INSTITUTE
FOR PROCESS ENGINEERING
AND PACKAGING IVV

Fraunhofer
IVV

EDITED BY
HORST-CHRISTIAN LANGOWSKI
AND
SVEN FRANKE

ORGANIZED BY

etv
European Technology Institute

IN COOPERATION WITH

Technische Universität München

EUROSIS-ETI Publications are ISI-Thomson and IET referenced
Legal Repository: Koninklijke Bibliotheek van België, Keizerslaan 4, 1000 Brussels, Belgium
CIP 12.620 D/2011/12.620/1

Selected papers of this conference are published in scientific journals.

For permission to publish a complete paper write EUROSIS, c/o Philippe Geril, ETI Executive Director, Greenbridge Science Park, Ghent University Ostend Campus, Wetenschapspark 1, Plassendale 1, B-8400 Ostend, Belgium.

EUROSIS is a Division of ETI Bvba, The European Technology Institute, Torhoutsesteenweg 162, Box 4, B-8400 Ostend, Belgium

Printed and bound in Belgium by Reproduct NV, Ghent, Belgium
Cover Design by Grafisch Bedrijf Lammaing, Ostend, Belgium

EUROSIS-ETI Publication
ISBN: 978-90-77381-72-4
EAN: 978-90-77381-72-4

A META-ANALYTICAL ASSESSMENT OF THE VARIABILITY BETWEEN ABATTOIRS IN THE EFFECT OF CHILLING ON THE *SALMONELLA* INCIDENCE ON PIG CARCASSES

Ursula Gonzales-Barron, James Sheridan, Francis Butler
UCD School of Biosystems Engineering
University College Dublin
UCD Belfield
Ireland
E-mail: ursula.gonzalesbarron@ucd.ie

Vasco Cadavez
Food Safety and Technology Group
CIMO Mountain Research Centre
Polytechnic Institute of Braganza
Portugal
E-mail: vcadavez@ipb.pt

KEYWORDS
Pig, slaughterhouse, meta-analysis, chilling, *Salmonella*.

ABSTRACT

The aim of this study was to estimate the overall effect of chilling on the *Salmonella* occurrence on pig carcasses during pork processing. Meta-analyses were conducted on the outcomes of research studies conducted in 32 abattoirs. A random-effects model was applied, and the between-abattoir variability (τ^2=0.548) was significant ($p<0.001$). Results also showed that publication bias is unlikely. Thus, this meta-analysis allowed the generalisation that chilling reduces the *Salmonella* incidence by a factor of 1.62 (95% CI: 1.09-2.41). However, a multilevel meta-analysis showed that the 'number of sampling visits', within abattoir, significantly ($p<0.05$) affected the chilling effect size, accounting for 40% of the between-abattoir variability. Thus, the beneficial effect of chilling only became apparent in a consistent way when at least two sampling visits took place in an abattoir.

INTRODUCTION

Meta-analysis concerns the statistical summarisation of the results of a large collection of independently conducted primary studies on one specific research question (Glass 1976). Thus, meta-analysis aims to explain differences in study outcomes by coding study characteristics such as research design features, data collection procedures or type of subjects sampled (Hox and de Leeuw 2003). In a fixed-effects model, the studies are regarded as simple replications of each other. This assumption considers that the possible differences between study outcomes are due to sampling error. However, heterogeneity in primary study outcomes is expected as different studies employ different experimental manipulations or measure the effects with different methodologies. To address this heterogeneity, a random-effects model is the best choice as it assumes that study outcomes vary not only because of random sampling effects (within-study variations), but also because of real differences between studies. If heterogeneity among primary studies is present, the next goal is then to identify the study characteristics or moderators that explain the differences between study outcomes. For this, an original new analytic direction was taken by Raudenbush and Bryk (1985), who argued that a meta-analysis could be regarded as a special case of *multilevel analysis* using hierarchical linear models, with subjects between studies at the first level and studies at the second level. The major advantage of using multilevel analysis instead of classical meta-analysis methods is its flexibility as it is simple to include moderators as explanatory variables in the model (Hox and Leeuw 2003).

In food safety research, meta-analysis may be conducted to address a broad range of research questions such as disease incidence, consumer practices, prevalence of microorganisms, effect of interventions pre- and post-harvest, etc. Performing a meta-analysis, Gonzales-Barron et al. (2008) confirmed that chilling has a significant effect on the reduction of pathogenic *Salmonella* occurrence in pig carcasses. However, the effect size of chilling was estimated using only pooled numbers of positive and tested carcasses per primary studied; being a case of a classical meta-analysis integrating only summarised outcomes. As other primary studies became available, it was realised that, for most of the studies, the *Salmonella* occurrence data of pigs pre and post chill could be broken down by abattoir. Thus, the objectives of this research were (i) to revise the effect size of chilling on *Salmonella* occurrence on pig carcasses considering the results of new primary studies; (ii) to quantify the between-abattoir variability in the effect of chilling; and (iii) to demonstrate the use of simple multilevel meta-analysis to attempt to explain the heterogeneity in the primary study outcomes.

METHODOLOGY

As indicated by Sargeant et al. (2005), to perform a meta-analysis, three important facets are to be considered: population, intervention or treatment and measured outcome. The problem statement in this study was the estimation of the overall effect of chilling on the prevalence of *Salmonella* on pig carcasses during slaughterhouse processing. The *population* was specified as eviscerated pig carcasses after veterinarian inspection in abattoirs. The *treatment* is represented by the chilling stage during carcass/pork processing, which includes cooling and cold storage (18 to 24 h) at ~5 C. The *measured outcome* is the detection of *Salmonella* on the pig carcass surface. Following the systematic review protocol presented by Sargeant et al. (2005), and after assessing all the

Table 1: Occurrence of *Salmonella*-Positive Pig Carcasses By Abattoir Before and After Chilling as Detected in Eleven Primary Studies

Primary study	Coded study	Coded Abattoir (j)	Number of visits (Nv)	Pre-chill group (Control)		Post-chill group (Treated)	
				s_C	n_C	s_T	n_T
Booteldorn et al. (2003)	1	1	2	14	55	9	55
		2	1	2	30	3	20
Bouvet et al. (2003)	2	3	2	6	60	3	60
		4	2	1	62	3	62
		5	2	1	60	0	60
Cutter (2003)	3	6	1	0	23	4	23
		7	1	0	30	2	30
		8	1	2	45	2	45
		9	1	0	30	0	40
		10	1	1	30	0	15
		11	1	1	15	0	15
		12	1	0	15	0	15
Davies et al (1999)	4	13	1	7	25	3	25
Dugan et al. (2010)	5	14	4	1	66	1	61
		15	3	12	50	2	47
		16	2	3	29	1	23
		17	3	2	30	1	30
Minvielle (unpublished data)	6	18	2	4	60	0	60
		19	2	8	60	4	60
		20	2	4	60	16	60
Oosterom et al. (1985)	7	21	NS[1]	19	64	9	64
		22	NS	4	71	2	71
		23	NS	4	75	1	75
Epling et al (1993)	8	24	NS	61	188	72	188
Algino et al. (2009)	9	25	NS	10	112	25	112
De Busser et al. (2011)	10	26	2	0	39	0	39
		27	2	2	43	0	43
		28	2	23	51	4	51
		29	2	5	44	1	44
		30	2	1	49	0	49
Arguello et al. (2011)	11	31	3	118	311	31	310
		32	3	61	135	17	135

[1] Number of visits within each sampled abattoir not specified

information presented in every study, eleven primary studies were considered appropriate for inclusion in the meta-analysis models (Table1). Next, a parameterisation or measure unit of the intervention's effect size needs to be determined. The effect size (θ) refers to the degree to which the hypothetical phenomenon is present in the population. Relative risk (RR) was chosen as the effect size parameterization for being less susceptible to differences in study protocols (Gonzales-Barron et al. 2008). The outcome data were available on n_T pig carcasses in the post-chill group (treated group) and n_C pig carcasses in the pre-chill group (control group). The number of successes (*Salmonella*-positive carcasses) in the post-chill and pre-chill group are represented by s_T and s_C, respectively. Results in Table 1 are presented broken down by abattoir surveyed (j), and includes the study characteristic or moderating variable of number of sampling visits per abattoir (Nv). A fixed-effects, random-effects and a multilevel model with the moderating variable Nv were fitted. The fixed-effects and random-effects model were fitted using the same data set presented in Table 1. However, as the number of visits per abattoir Nv were not available in Oosterom et al. (1985), Epling et al. (1993) and Algino et al. (2009), the results from these three primary studies were removed for the multilevel model.

Fixed-effects meta-analysis

In its simplest form, a fixed-effects approach can be carried out to make a *conditional inference* only about the j primary studies included in the meta-analysis (Hedges and Vevea 1998). On the other hand, a fixed-effects meta-analysis can also be conducted under the assumption that the possible differences between study results are only due to sampling variance. In any case,

$$\theta_j = \Theta + \varepsilon_j \qquad (1)$$

with θ_j the observed effect size in the abattoir j, Θ the population effect size, and ε_j the residual error due to sampling variance. It is assumed that the ε_j have a normal distribution with mean zero and a true variance ξ^2. In our particular case, the effect size θ_j refers to the natural logarithm of the relative risk (log RR). Apart from the values of θ_j from each abattoir, the standard error of the effect size $\sigma(\theta_j)$ are to be calculated.

$$\theta_j = \log RR = \log \frac{s_T / n_T}{s_C / n_C} \tag{2}$$

$$\sigma(\theta_j) = \sigma(\log RR) = \left(\frac{n_T - s_T}{n_T s_T} + \frac{n_C - s_C}{n_C s_C} \right)^{0.5} \tag{3}$$

To estimate the population effect size Θ, the observed size effects θ_j should be averaged. Because studies usually differ in the reliability of estimating the true effect size, for instance, due to differences in study size, a weighted average is preferred with weights w_j equal to the precision in estimating the population effect size. Because in this simple fixed-effects model, it is assumed that the deviation of the observed effect sizes from the population effect size is due to sampling error alone, the precision can be defined as the inverse of the (estimated) sampling variance. The estimated population effect size and its standard error would be,

$$\hat{\Theta} = \frac{\sum_j w_j \theta_j}{\sum_j w_j} \tag{4}$$

$$\hat{\sigma}(\hat{\Theta}) = \frac{1}{\left(\sum_j w_j \right)^{0.5}} \tag{5}$$

with $w_j = 1/\sigma^2(\theta_j)$. Because meta-analyses are performed retrospectively, in many situations studies may differ from each other due to differences in measuring protocols, in the population from which the sample is drawn, and in the kind of dose and treatment that is offered; all of these giving rise to heterogeneity. A popular homogeneity test is the Q statistic,

$$Q = \sum_j \frac{(\theta_j - \hat{\Theta})^2}{\sigma^2(\theta_j)} \tag{6}$$

When effect sizes across studies are homogeneous, Q follows a chi-square distribution with $(j\text{-}1)$ df. If the hypothesis is rejected, there is evidence that there are additional sources of variability other than within-study sampling error. It is then common practice to examine moderator variables; divide the studies in homogeneous groups to perform separate meta-analysis; or to use a random-effects model.

Random-effects meta-analysis

One way to model the heterogeneity among the true effects measured by the primary studies is to treat it as purely random (Viechtbauer 2010). In contrast to the fixed-effects model, random models provide an *unconditional inference* about a larger set of studies from which the j studies included in the meta-analysis are assumed to be a random sample (Hedges and Vevea 1998). In a random-effects model, each study investigates its own true effect size Θ_j,

$$\theta_j = \Theta_j + \varepsilon_j = \overline{\Theta} + v_j + \varepsilon_j \tag{7}$$

with $\overline{\Theta}$ the mean true effect size and v_j the deviation of the true study effect size from the mean true effect size. The values of v_j are normally distributed random effects with a mean of zero and a variance of τ^2. In this approach, two sources of variation are distinguished: sampling variation (ξ^2) and variation between true effect sizes (τ^2). By including τ^2, the standard error in the effect size estimates represents random variability at both the subject level and the study level. As in the fixed-effects approach, a weighted method is also used to estimate the mean true effect size and its standard error. The inverse variance weight or precision of the primary studies should then be corrected (w^*_j) by addition of the between-study variability term τ^2,

$$w^*_j = \frac{1}{\sigma^2(\theta_j) + \tau^2} \tag{8}$$

with the variance τ^2 estimated from the Q-statistic,

$$\hat{\tau}^2 = \frac{Q - (j-1)}{\sum_j w_j - \left(\sum_j w_j^2 \Big/ \sum_j w_j \right)} \tag{9}$$

The mean true effect size Θ and its standard error $\sigma(\Theta)$ are estimated with equations (4) and (5) using instead the corrected weights w^*_j.

Multilevel meta-analysis

A meta-analysis can be considered a special case of *multilevel analysis* using hierarchical linear models, with subjects between studies at the first level and studies at the second level. If the between-study variance is shown to be noteworthy, one or more moderators (study characteristics) can be added to the model to account for at least part of the heterogeneity in the true effects. In our model, the moderator Nv (number of sampling visits in an abattoir) was added,

$$\theta_j = \Theta_j + \varepsilon_j = \beta_0 + \beta_1 Nv_j + v_j + \varepsilon_j \tag{10}$$

This model treats the moderator effect β_1 as fixed and the v_j as random effects that distribute normally with a mean of zero and a variance of τ^2. Yet, τ^2 now denotes the amount of residual heterogeneity among the true effects, or the variability among the true effects that is not accounted for by the moderators included in the model. To estimate the parameters, maximum likelihood estimation (MLE) procedures are the most frequently used. In MLE, residuals on both levels (v_j and ε_j of Equation 10) are assumed to be independently distributed. Meta-analysis models were fitted in R version 2.14.2 (R Development Core Team) using the 'metafor' package (Viechtbauer 2010), which provides functions for fitting the models described above.

RESULTS AND DISCUSSION

The fixed-effects model provided strong evidence that the chilling stage in pork processing has a decreasing effect on the occurrence of *Salmonella* on pig carcasses ($p<0.001$). After taking the inverse of the exponential of the estimated overall effect size (Θ=-0.550 in Table 2), it can be inferred that, on average, chilling reduces the *Samonella* incidence by a factor of ~1.73 (95% CI: 1.49 – 2.02). Although there is evidence of heterogeneity by the significant Q statistic (Table 2), this fixed-effects model still provides valid inferences as long as they are restricted to the set of studies included in the meta-analysis (Viechtbauer 2010). The forest plot shown in Figure 1 highlights the variability in effect size estimates and precision between abattoir entries; and the marker size illustrates the contribution of each abattoir (weight) to the overall effect estimate for the fixed-effects solution. It should be noticed that weights are not only related to sample size, but instead to the number of successes or failures in proportion to the sample size. Analyzing the definition of the standard error of the Ln RR parameterization (Equation 3), inverse-variance weights will be small when the number of *Salmonella*-positive carcasses (successes) in either group (before or after chilling) is close to zero. For instance, abattoirs 26 and 30, with a reduced number of successes pre-chill and post-chill (Table 1) produced very low weights (Figure 1). In contrast, weights will be large when the number of successes in both groups are high. In an extreme case, where there was no failure in both groups, the weights would be equal to infinity. That explains why abattoirs 24, 31 and 32 with the highest sample sizes and the highest proportion of successes (Table 1) produced the highest weights (Figure 1). Yet, small studies can still be given a large weight when they have relatively more successes. For instance, abattoir 1 with a sample size (n_C=n_T=55) smaller than abattoir 5 (n_C=n_T=60) was assigned a higher weight (Figure 1) since its number of successes in proportion to sample size was much higher than the latter (Table 1). Thus, the inverse-variance weighting permits the consideration of small but well-designed studies, that otherwise would have been disregarded because of their lack of statistical power to show a significant different for the outcome of interest, had the weighting been based on sample size alone.

A visual examination of the forest plot (Figure 1) gives an idea of the discrepancy among abattoir entries, which is not surprising given the several sources of variability among studies and abattoirs such as sampling site, chilling equipment, cross contamination of carcasses, level of *Salmonella* infection at slaughterhouses, differences in the microbiological protocol, year and country, among others. In the random-effect model, the overall effect size is still significant although at a limiting p-value (Table 2). This occurred because the addition of the relatively high variability at abattoir level to the random variability at carcass level, produced an increase of the standard error of the overall effect size from 0.077 (fixed-effects) to 0.201 (random-effects; Table 2). The overall effect size is still significant but lower than the one estimated by fixed effects, and suggests that after chilling the *Salmonella* prevalence is reduced on average by a factor of ~1.62 (Θ=-0.485 in Table 2). Accounting for the heterogeneity in the true log relative risks between abattoirs (τ^2=0.548) led to a meta-analysis with better fit quality than the fixed-effects model, as indicated by the lower log-likelihood and Bayesian Information Criterion (BIC).

Table 2: Results of the Abattoir-Level Meta-Analysis Models for the Risk of *Salmonella* Prevalence on Pig Carcasses After Chilling Relative to Before Chilling

Model	Fixed-effects	Random-effects	Multilevel
Parameters			
Intercept (Θ, β_0)	-0.550 (0.077)***	-0.485 (0.201)*	0.607 (0.612) ns
# visits in abattoir (β_1)			-0.589 (0.273)*
Heterogeneity			
Q test	107.5 (df=31)***		
τ^2		0.548	0.331
I^2		68.3%	
H^2		3.15	
QM moderators			4.66 (df=1)*
QE residual heterog.			34.05 (df=25) ns
Goodness-of-fit			
Log-likelihood	-78.2882	-49.6887	-41.2337
BIC	160.0421	106.2453	92.1240

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' Non-significant 'ns'

Other measures can be computed to facilitate the interpretation of the estimated amount of between-abattoir heterogeneity. The I^2 statistics or intra-class correlation estimates the proportion of between-study variance from the total variance. This is analogous to using the proportion of explained variance in standard regression models to indicate the importance of specific predictor variables. Hunter and Schmidt (1990) pointed out that with a large number of studies, the power of the significance test is high, and small variances will become significant. However, when the number of studies is small, lack of significance for τ^2 does not imply that the outcome are homogeneous. So, theypropose a 25% rule of thumb; this is, if the intra-class variance is higher than 25% of the total variance, the variance between studies can be deemed as large enough to attempt to model it using available study characteristics. In our case, the intra-class correlation (I^2=68.3%) underscored the presence of between-abattoir variance, and that consequently some study characteristics could be coded to attempt to explain such heterogeneity among the true effects. The H^2 statistic is the ratio of the total amount of variability in the observed outcome to the amount of sampling variability (If τ^2=0, then H^2=1). In this model, the H^2 ratio was 3.15 (Table 2). In contrast with the fixed-effects solution, the overall effect size – or incidence reduction ratio due to chilling (95% CI: 1.09 – 2.41) – obtained by the random-effects approach can be generalised beyond the specific set of abattoirs at hand, and this is also sustained by the unlikely presence of publication bias. This will be later discussed.

The power of multilevel meta-analysis becomes apparent when attempting to model the significant differences in the abattoir outcomes. We hypothesised that as an abattoir was visited more number of times (i.e., sampled more), the effect size of chilling on *Salmonella* occurrence became more clear and larger; and that at least part of the heterogeneity found between abattoirs may be due to the influence of the number of sampling visits (Nv) defined in the experimental design of the primary studies. The results of the multilevel

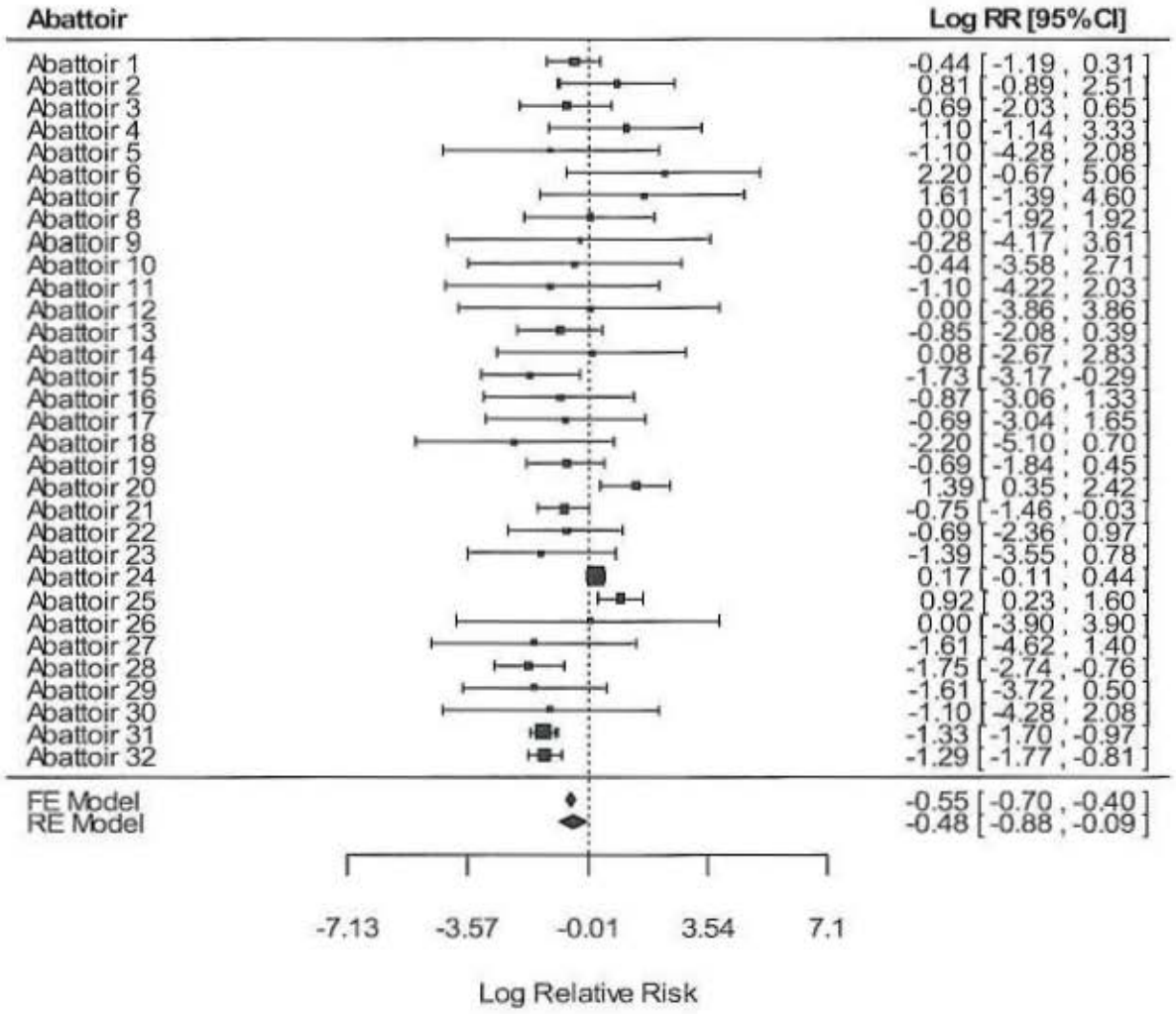

Figure 1: Forest Plot of the Risk of *Salmonella* Prevalence on Pig Carcasses After Chilling relative to Before Chilling. Primary Study Estimates and Overall Fixed and Random Effects are shown with 95% Confidence Intervals

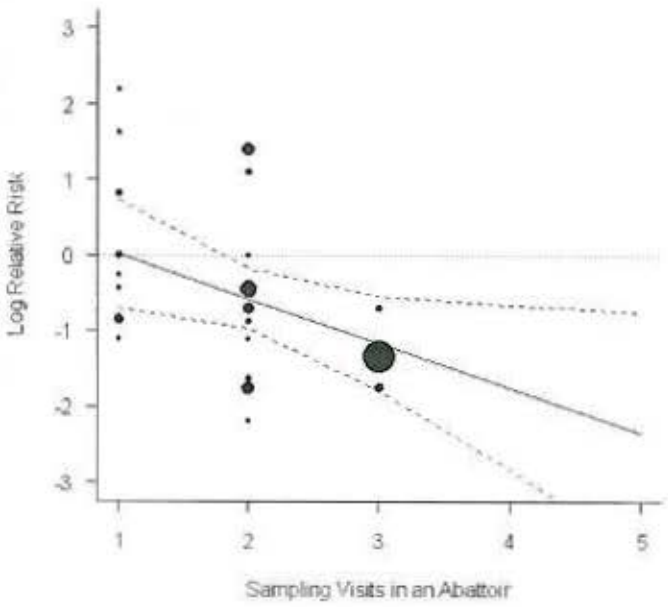

Figure 2: Effect of the Number of Sampling Visits in a Study Experimental Design on the Estimation of the Effect of Chilling on *Salmonella* Incidence on Pig Carcasses

model (Table 2) showed that, apparently, our estimate of the effect size of chilling is subject to the number of times an abattoir is visited. This is supported by the significant coefficient β_1 and the significant QM test for the moderating variable. In the multilevel model, the estimated amount of residual heterogeneity is τ^2=0.331, suggesting that 39.6% (0.548-0.331)/0.548) of the total amount of heterogeneity between abattoirs can be accounted for by including the 'number of sampling visits' in the model. The QE statistic indicates that the residual heterogeneity is no longer significant. However, we can assume that the number of sampling visits per abattoir may be accounting for some of the between-abattoir variability, and that other non-coded study characteristics may be also noteworthy.

The value of the coefficient β_1 indicates that an increase in one sampling visit in abattoir corresponds to a change of -0.589 units in terms of the average log relative risk (Table 2). Because the abattoir outcome depends on the number of sampling visits, reporting an overall effect size does not convey the relevant information. To facilitate the interpretation of the moderator, predicted average log relative risks for different number of sampling visits can be reported. Figure 2 shows a plot of the log RR as a function of the number of sampling visits. The observed RR are drawn proportional to the weights, and predictions are shown with corresponding 95% confidence interval bounds. Results suggest that when an abattoir is sampled only once, the observation of an effect (either increasing or decreasing) is inconsistent, and therefore, on average no effect is observed (notice that on average the Log RR is zero when one sampling visit is performed). Seemingly, the decreasing effect of chilling on the *Salmonella* prevalence becomes noticeable (and significant) with at least two sampling visits. These interesting results may be explained by the fact that, although *Salmonella* viability has been proven, at least at laboratory level, to be affected by both temperature (cold shock and refrigerated storage) and water activity (osmotic shock); still the efficacy of the chilling operation for the reduction of *Salmonella* is also affected by other equally important factors, related to chilling systems, abattoir logistics, cross-contamination, abattoir hygiene, etc. In addition, *Salmonella* cells are not homogeneously distributed on carcasses, which will greatly add to the *uncertainty* in the measured outcomes (i.e., although a pre-chill carcass may contain *Salmonella* cells, swabbing a *Salmonella*-free area will lead to a negative result). On the other hand, the pre-chill and post-chill measurements were most of the times performed on different carcasses, which adds extra randomness to the measured outcome. Thus, it is then expected that, with so many factors affecting the efficacy (and the measurement itself of the efficacy) of the chilling operation, results from only one abattoir visit made up of an average of 30 pig carcasses, will not be sufficient to consistently elucidate any effect. Results from the multilevel meta-analysis indicated that a significant reduction ratio (~1.77) of chilling on the *Salmonella* incidence on pig carcasses was observed with at least two sampling visits consisting of a total of 50 carcasses on average. A greater *Salmonella* incidence reduction ratio (~3.20) was noticed with data from three sampling visits (Figure 2). This meta-analysis model however is not supposed to be extrapolated for four or more sampling visits, as the number of sampling ocassions is not a continuous variable but a categorical one.

An important problem in meta-analysis is the so-called file drawer problem or publication bias. The data for a meta-analysis are the results from previously published studies. Studies that find significant results may have a larger probability to be published. As a result, a sample of published studies can be biased in the direction of reporting large effects. An approach to investigate publication bias is by means of a funnel plot which is a plot of the effect sizes versus their standard errors. If the sample of available studies is 'well behaved', this plot should have the shape of a funnel. The outcomes from smaller studies (normally of higher standard errors) are more variable but estimate the same underlying population parameter. If large effects are found predominantly in smaller studies, this indicates the possibility of publication bias, and the possibility of many others insignificant small studies remaining unpublished (Hox and De Leeuw 2003). In the case of multilevel models with moderating variables, the funnel plot should not be based on the observed outcomes (i.e., effect sizes versus standard errors) because part of the variability in the plot could be due to the explanatory study characteristics. Thus, it is more appropriate to use a funnel plot after removing the covariate effects. Figure 3 shows a funnel plot of the effect size residuals against their corresponding standard errors, and does not suggest evidence of publication bias.

CONCLUSION

Both the fixed-effects and random-effects model confirmed the effect of chilling in decreasing *Salmonella* incidence; although the random-effects model was preferred to account

for the high variability observed among the 32 abattoirs surveyed in the 13 primary studies. As a funnel plot suggested no evidence of publication bias, it can be safely generalised that the chilling effect reduces the incidence of *Salmonella* by a factor of 1.62 (95% CI: 1.09 – 2.41) in relation to the occurrence in pre-chill pig carcasses. The heterogeneity in effect size between surveyed abattoirs (τ^2=0.548), investigated by means of a multilevel model, revealed that the 'number of sampling visits' performed in an abattoir is a study characteristic that has a major influence on the estimated chilling effect. This moderating variable (covariant) explained 40% of the between-abattoir variability, and revealed that only one sampling visit – consisting of an average of 30 carcasses – was not sufficient to consistently elucidate any chilling effect; most likely because of the many factors influencing both the efficacy of the chilling operation (i.e., chilling systems, abattoir logistics, proximity between carcasses, cross-contamination, etc.) and the measurement itself (i.e., heterogeneous distribution of bacterial cells on carcasses; uncertainties associated with the different carcasses sampled before and after chilling; and with the microbial test protocol). The beneficial effect of chilling became only evident in a consistent way with at least two sampling visits per abattoir; this is with higher sample sizes that could surmount the different sources of variability and uncertainty affecting the efficiency of the chilling operation. Meta-analysis applications such as the one conducted in this study are of importance in the development of risk assessment models; in the design of future statistically-sound incidence surveys in abattoirs, and ultimately in the compilation and better understanding of the differing outcomes found by primary studies.

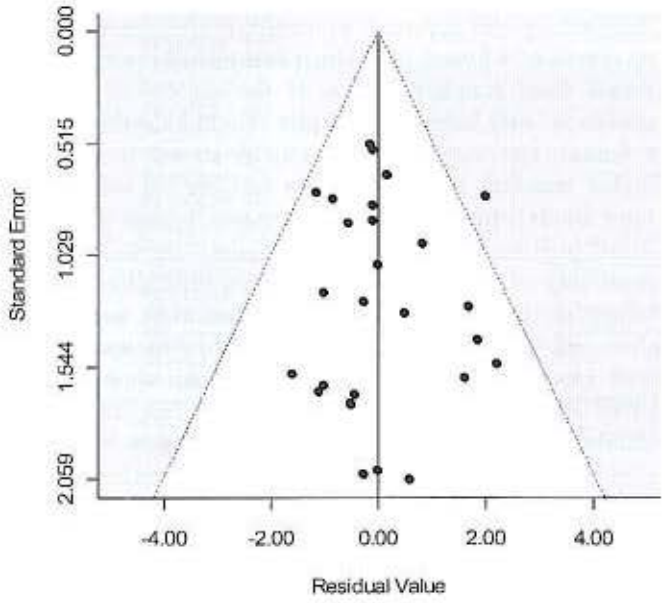

Figure 3: Funnel Plot of the Residuals of the Risk of *Salmonella* Prevalence on Pig Carcasses After Chilling relative to Before Chilling

REFERENCES

Algino R. J; G. A. Badtram ; B. H. Ingham; and S. C. Ingham. 2009. "Factors Associated with Salmonella Prevalence on Pork Carcasses in Very Small Abattoirs in Wisconsin". Journal of Food Protection 72, No. 4 (Apr), 714-721.

Arguello, H. ; A. Carvajal; J. A. Collazos; C. Garcia-Feliz; and P. Rubio. 2012. "Prevalence and Serovars of *Salmonella* enterica on Pig Carcasses, Slaughtered Pigs and the Environment of Four Spanish Slaughterhouses". Food Research International 45, No. 2 (Mar), 905-912.

Botteldoorn, N.; M. Heyndrickx; N. Rijpens; K. Grijspeerdt; and L. Herman. 2003. "*Salmonella* on Pig Carcasses: Positive Pigs and Cross Contamination in the Slaughterhouse". Journal of Applied Microbiology 95, 891-903.

Bouvet, J.; C. Bavai ; R. Rossel ; A. Le Roux ; M. P. Montet ; C. Mazuy ; and C. Vernozy-Rozand. 2003. "Evolution of Pig Carcass and Slaughterhouse Environment Contamination by *Salmonella*". Reviews Veterinary Medicine 154, No. 12 (Dec), 775-779.

Cutter, C. 2003. "Effects of Commercial Chilling Methods for Reducing Bacteria on Pork Carcasses". In *Research Report Pork Safety*. National Pork Board, Des Moines, Iowa.

Davies, R. H.; I. M. McLaren; and S. Bedford. 1999. "Distribution of *Salmonella* Contamination in Two Pig Abattoirs" In *Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork* (Washington DC, USA) 267-272.

De Busser, E.V.; D. Maes; K. Houf; J. Dewulf; H. Imberechts; S. Bertrand; and L. De Zutter. 2011. "Detection and Characterization of *Salmonella* in Lairage, on Pig Carcasses and Intestines in Five Slaughterhouses". International Journal of Food Microbiology 145, 279–286

Duggan S. J.; C. Mannion; D. M. Prendergast; N. Leonard; S. Fanning; U. Gonzales-Barron; J. Egan; F. Butler; G. Duffy. 2010. "Tracking the *Salmonella* Status of Pigs and Pork from Lairage through the Slaughter Process in the Republic of Ireland". Journal of Food Protection 73, No. 12 (Dec), 2148-2160.

Epling, L. K.; J. A. Carpenter; and L. C. Blakenship. 1993. "Prevalence of *Campylobacter* spp. & *Salmonella* spp. on Pork Carcasses and the Reduction Affected by Spraying with Lactic Acid". Journal of Food Protection 56 ,No. 6 (Jun), 536–537.

Glass, G. V. 1976. "Primary, Secondary and Meta-analysis of Research". Educational Researcher 5 (1, 3): 3-8.

Gonzales Barron, U.; D. Bergin; and F. Butler. 2008. "A Meta-analysis Study of the Effect of Chilling on *Salmonella* Prevalence on Pork Carcasses". Journal of Food Protection 71, No. 7 (Jul), 1330-1337.

Hedges, L. V.; and J. L. Vevea. 1998. "Fixed- and Random-Effects Models in Meta-Analysis. Psychological Methods 3, 486-504.

Hox, J. J.; E. De Leeuw. 2003. "Multilevel Models for Meta-Analysis". In *Multilevel Modelling: Methodological Advances, Issues and Applications*, S.P. Reise and N. Duan (Eds.). Lawrence Erlbaum Associates, NJ, 90-111.

Hunter, J. E; and F. L. Schmidt. 1990. „Methods of Meta-Analysis: Correcting Error and Bias in Research Findings". Sage, Newbury Park, CA.

Oosterom, J.; R. Dekker; G. J. A. de Wilde; F. van Kempen-de Troye; and G. B. Engels. 1985. "Prevalence of *Campylobacter jejuni* and *Salmonella* during pig slaughtering". Veterinary Quarterly 7, No. 1 (Jan), 31-34.

Raudenbush, S. W.; A. S. Bryk. 1985. "Empirical Bayes Meta-Analysis". Journal of Educational Statistics 10, 75-98.

Sargeant, J. M.; M. Amezcua; A. Rajic; and L. Waddell. 2005. "A guide to conducting systematic reviews in agri-food public

health". Food Safety Research and Response Network, Canada.
Viechtbauer, W. 2010. "Conducting Meta-Analyses in R with the 'Metafor' Package". Journal of Statistical Software 36, No. 3 (Aug), 1-48.

BIOGRAPHIES

Dr. URSULA GONZALES BARRON is a senior researcher at the School of Biosystems Engineering, University College Dublin (UCD), Ireland. Her expertise resides in the mathematical aspects of food safety such as risk assessment modelling and predictive microbiology. Other interests are the use of meta-analysis and Bayesian statistics in food safety, zero-modified count data models for microbial counts, acceptance sampling and statistical process control for microbial load, as well as food traceability.

Dr. VASCO CADAVEZ is an Adjunt Professor at the Department of Animal Science of the Polytechnic Institute of Braganza (IPB), Portugal. His research interests include carcass and meat quality, with special emphasis on the development of mathematical models for the prediction of carcasses composition. Since 2009, he acts as Principal Investigator of the Food Safety and Technology Research Group at the Mountain Research Centre (CIMO), IPB.