Meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their products

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A B S T R A C T

Meat and meat products are the main vehicles of foodborne diseases in humans caused by pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, verotoxigenic *Escherichia coli* (VTEC) and *Staphylococcus aureus*. In order to prioritise research on those microbial hazards, a meta-analysis study was conducted to summarise available information on the presence of such pathogens in meats produced in Portugal. By using a logit-transformed proportion as effect size parameterisation, a number of multilevel random-effect meta-analysis models were fitted to estimate mean occurrence rates of pathogens, and to compare them among meat categories (i.e., bovine meat, broiler meat, pork, minced beef and minced pork), and among meat product categories (i.e., intended to be eaten cooked, to be eaten raw and cured meats). The mean occurrence rate of *Campylobacter* in Portuguese broiler meat (40%; 95% CI: 22.0–61.4%) was about ten times higher than that of *Salmonella* (4.0%; 95% CI: 1.4–10.8%); although these levels were comparable to current EU ranges. Nevertheless, in the other meat categories, the meta-analysed incidences of *Salmonella* were slightly to moderately higher than EU averages. A semi-quantitative risk ranking of pathogens in Portuguese-produced pork pointed *Salmonella* spp. as critical (with a mean occurrence of 12.6%; 95% CI: 8.0–19.3%), and *Y. enterocolitica* as high (6.8%; 95% CI: 2.2–19.3%). In the case of the Portuguese meat products, the non-compliance to EU microbiological criteria for *L. monocytogenes* (8.8%; 95% CI: 6.5–11.8%) and *Salmonella* spp. (9.7%; 95% CI: 7.0–13.4%) at sample units level, in the categories 'intended to be eaten cooked' and 'to be eaten raw', were considerably higher than EU levels for ready-to-eat products in comparable categories. *S. aureus* was the pathogen of greatest concern given its high occurrence (22.6%; 95% CI: 15.4–31.8%) in meat products. These results emphasised the necessity of Portuguese food safety agencies to take monitoring, and training actions for the maintenance of good hygiene practices during the production of the great variety of traditional meat products. This meta-analysis study also highlighted important gaps of knowledge, and may assist food safety authorities in the prioritisation of microbiological hazards, and the implementation of essential food safety assurance systems at primary production.

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1. Introduction

Raw meat provides an ideal growth medium for a wide range of pathogens, and, if there is any malpractice in the handling, post-processing, storage or cooking of the product, illness can be a real possibility. Contamination of meat with foodborne pathogens is a major public health issue. In fact, in 2011, campylobacteriosis was the most commonly reported gastrointestinal bacterial pathogen in humans in the EU, followed by salmonellosis, with 220,209 and 95,548 confirmed cases, respectively While campylobacteriosis has increased significantly over the past four reported years (2008–2011), salmonellosis continues its decreasing trend since 2007 although it is the most reported cause of outbreaks (EFSA, 2013). In particular, the human cases caused by the two most common serovars, *Salmonella* Enteritidis (44%) and *Salmonella* Typhimurium (25%), diminished significantly since 2008. In foodstuffs, the highest proportion of *Campylobacter* positive samples was once again reported for fresh poultry (31.3% of positive samples), while *Salmonella* serovars were most often detected in fresh broiler (6.7%) and pig meat (0.7%). Furthermore, non-compliance with the EU *Salmonella* criteria has been most often observed in foods of meat origin, being higher for minced poultry (6.8% based on sample units) and minced meat from other species (1.1%) intended to be eaten cooked and minced meat and meat preparations intended to be eaten raw (1.6%) (EFSA, 2013).

In the case of the verotoxigenic *Escherichia coli* (VTEC) infections (9485 cases), a 2.6-fold increase was observed in comparison to 2010, while the confirmed EU cases of human listeriosis (1476) was slightly lower than previous years, yet with a high fatality rate of 12% (EFSA, 2013). Contaminated bovine meat (1.4% of contaminated sample units in 2011) continues to be considered the major source of VTEC infections in humans (EFSA, 2012, 2013), while non-compliance with the EU *Listeria monocytogenes* criteria is mostly observed in ready-to-eat (RTE) fishery products (6.7%) and of meat origin (2.4%). In 2011, although following a decreasing five-year trend, yersiniosis was the fourth most frequently reported zoonosis (7017 confirmed cases) in

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the EU. Pigs are considered to be a major reservoir while pork and poultry products are considered to be the most important source of pathogenic *Yersinia enterocolitica* infection in humans (Simonová, Vazlerová & Steinhauserová, 2007). In fact, in pig meat samples taken from four European countries, the overall incidence of *Y. enterocolitica* was 2.4% (EFSA, 2013).

In Portugal, there is considerably less information on zoonoses, and incidence of pathogens in meats. Because of the current compulsory national surveillance, and control programmes of *Salmonella* in foods, more information is available for this pathogen. According to the last EFSA report (EFSA, 2013), the notification rates of salmonellosis in 2011 (1.6 confirmed cases per 100,000) appeared lower than the EU average (20.7 per 100,000), and, peculiarly, well below other Western European countries such as Spain (32.8 per 100,000), France (13.4 per 100,000) and Denmark (21.0 per 100,000). However, as Portugal has one of the highest hospitalisation rates (84% as pointed out in the same report), this may indicate that, apart from a likely large number of citizens not seeking medical advice, there is also under-reporting by the surveillance systems which capture primarily the most severe cases. With regard to the foodstuff contaminated with *Salmonella* spp. in Portugal, pig meat has been identified as the most important likely source of infection, with a mean incidence of 5.0%, in comparison with the EU average of 0.6% (EFSA, 2013). Nevertheless, information with regard to other foodborne diseases is scarce (i.e., no Portuguese surveillance systems in place for campylobacteriosis, listeriosis, yersiniosis and VTEC infections), which leads to an inaccurate evaluation of the relative importance of each foodborne disease. Due to the limited zoonosis information, it is difficult to establish an evolution trend of the incidence of foodborne diseases as well as the occurrence of the main microbial contaminants in Portuguese foods in the last years (Veiga et al., 2012). Nonetheless, given (i) the strong association of foodborne diseases in humans with the consumption of contaminated meat and meat products, and (ii) the high consumption of meats (93 kg per Portuguese habitant in 2012 above the average 80 kg per EU citizen) and meat products (672 tonnes production in 2009 in Portugal), it is imperative to gather as much information as possible on the levels of foodborne pathogens in Portuguese meats and meat products in order to understand the current epidemiological situation, prioritise microbial hazards for risk analysis, and identify knowledge gaps to provide direction for further research.

Meta-analysis is a body of summarising statistical techniques whose objective is to synthesise, integrate and contrast the results from a large amount of primary studies investigating the same research question (Gonzales-Barron, Cadavez, Sheridan, & Butler, 2013). The primary objective of meta-analysis is to produce a more precise estimate of the effect size of a particular treatment, with increased statistical power, than is possible using only a single study (Sutton, Abrams, & Jones, 2001). Yet, with meta-analysis, it is also possible to explain differences in the study outcomes by coding study characteristics, such as: research design features, data collection procedures, type of samples or even year (Hox & De Leeuw, 2003). In the past few years, meta-analysis has increasingly been applied in food safety (Den Besten & Zwietering, 2012; Gonzales Barron, Bergin, & Butler, 2008; Gonzales-Barron & Butler, 2011; Gonzales-Barron et al., 2013; Grieg et al., 2012; McQuestin, Shadbolt, & Ross, 2009; Sánchez, Dohoo, Christensen, & Rajic, 2007). In food safety research, meta-analysis may be conducted to address a broad range of research questions such as disease incidence, prevalence of microorganisms in foods, effect of interventions pre- and post-harvest, risk ranking of pathogens and consumer practices, among others. Thus, the objectives of this research are: (i) to compile all publicly accessible information on the occurrence of *Salmonella* spp., *Campylobacter*, *L. monocytogenes*, VTEC, *Y. enterocolitica* and *Staphylococcus aureus* in Portuguese meats, and meat products grouped by categories; (ii) to quantitatively summarise, and compare the occurrence of pathogens according to available information by conducting separate meta-analysis models for meat and meat products; (iii) to appraise likely publication

bias, a common artefact in meta-analysis studies (Viechtbauer, 2010); (iv) to conduct a semi-quantitative risk ranking of pathogens in pork using the characterisation of severity of hazards proposed in EFSA (2011a); and (iv) to identify knowledge gaps on the occurrence of pathogens in certain meat categories.

2. Methodology

The *problem statement* in this study was the estimation of the overall incidence or occurrence of foodborne pathogens in Portuguese meats. The *population* was specified as meat and meat products produced in Portugal while the *measured outcome* is the detection of pathogens in meats sampled either at processing plants or at retail. Following the systematic review protocol presented by Sargeant, Amezcua, Rajic, and Waddell (2005), electronic searches were carried out to identify official reports published by national and international organisations (such as World Health Organisation, WHO; European Food Safety Authority, EFSA; International Commission for Microbiological Specification in Foods, ICMSF) reporting occurrence values of *Salmonella* spp., *Campylobacter*, *L. monocytogenes*, VTEC, *Y. enterocolitica* and *S. aureus* in Portuguese meats (categorised as: fresh bovine, fresh broiler, fresh pork, minced beef and minced pork) and meat products (categorised as: intended to be eaten raw, intended to be eaten cooked, and cured meats). Literature search to identify suitable scientific articles was conducted using the ISI Web of Knowledge and Web of Science databases for papers indexed since 1990 as well as Google searches using both English and Portuguese terms for combinations of foodborne disease or zoonosis (e.g., salmonellosis) or the pathogen (e.g., *Salmonella*), and the meat under study (e.g., pig meat, pork, pork product, pork preparation, sausage). For inclusion in the meta-analyses, the papers had to meet two requirements: to be an original article, and to make use of an approved microbiological method for pathogen detection.

Following the formulation of the problem statement and data collection, a parameterisation or measure unit of the effect size needs to be determined. The parameter measuring the effect size is a common metric that permits direct comparison and summation of primary studies (Noble, 2006). The effect size (θ) refers to the degree to which the hypothetical phenomenon (i.e., pathogens in meats) is present in the population. Because the measured outcome is binary (i.e., a meat sample tests either positive or negative for the pathogen) and is given only for single groups, the only possible parameter to measure effect size is the *raw proportion p* (or incidence) and its *transformations*. In order to restrict the range of the effect size or pathogen's incidence to [0–1] and to stabilise the variance, the logit transformation of the raw proportion was used as the effect size measure θ (Viechtbauer, 2010). If the sample size n of the primary study is at least higher than 20, it is usually reasonable to assume that the sampling distribution of the outcomes is normal (Bryk & Raudenbush, 1992).

2.1. Description of data sets

After assessing all the information presented in every study, a total of 21 primary studies – encompassing international reports and scientific articles – were considered appropriate for inclusion in the meta-analysis models. The meta-analysis models for fresh meats were based on 16 primary studies (Antunes, Reu, Sousa, Pestana, & Peixe, 2002; Baptista, 2010; Borges, 2009; EFSA, 2005, 2006, 2007, 2009, 2010a, 2010b, 2011b, 2012, 2013; Esteves, Aymerich, et al., 2006; Esteves, Saraiva, Fontes, & Martins, 2006; Mena et al., 2004; Mena, Rodrigues, Silva, Gibbs, & Teixeira, 2008), while the ones for meat products were based on 7 primary studies (Almeida, Mena, & Carneiro, 1998; Esteves, Aymerich, et al., 2006; Esteves, Patarata, Saraiva, & Martins, 2008; Esteves, Saraiva, et al., 2006; Ferreira, Fraqueza, & Barreto, 2007; Mena et al., 2004; Mendes, 2013; Vaz-Velho, Almeida, Mena, Carneiro, & Freitas, 1998). From each of the primary studies (j), the number of samples (s) experiencing the event of interest (i.e., testing positive for a

Table 1
Number of incidence and observations (s/n) of food-borne pathogens in Portuguese fresh meats by category extracted from published survey studies.

Pathogen	Fresh bovine	Fresh broiler	Fresh pork	Minced beef	Minced pork
Salmonella	3	7	12	2	5
	(11/180), (0/55), (0/1142)	(1/81), (4/216), (0/25), (2/50), (0/40), (44/421), (7/64)	(3/60), (25/105), (6/58), (2/61), (5/30), (62/1122), (39/256), (14/99). (16/99), (25/105), (13/101), (10/64)	(0/95), (3/135)	(13/130), (2/73), (5/186), (2/142), (3/120)
Campylobacter	0	8	0	0	0
		(17/81), (21/108), (0/33), (296/421), (21/78), (39/78), (46/62), (24/38)			
Y. enterocolitica	0	0	3	0	4
			(0/61), (0/58), (1/61)		(13/25), (10/25), (2/75), (0/74)
VTEC	0	0	3	0	1
			(0/74), (0/34), (4/25)		(5/50)
L. monocytogenes	1	2	0	0	0
	(3/17)	(26/63), (9/15)			
S. aureus	0	1	1	0	0
		(28/64)	(32/64)		

pathogen) and the total number of samples (n) were extracted. Information such as sample weight, production stage, sampling site of the carcass (if applicable) and year of the survey was also annotated from every primary study. For the Portuguese fresh meats, a total of fifty-one observations of incidence (s/n) of foodborne pathogens were extracted from the 16 primary studies, and are compiled according to meat categories in Table 1.

In the case of the Portuguese meat products, sixty-nine incidence observations on pathogens were found for 26 types of products. These were mainly traditional meat products elaborated with pig meat and fat, and were categorised as traditional products 'to be eaten raw' (encompassing different kinds of dry-fermented sausages) and 'to be eaten cooked' (including fresh sausages and blood sausages). A third category of 'cured meat products' was considered so as to comprise heat-treated meat products such as cooked sliced ham and *mortadella*. These three meat product categories are mutually exclusive, and the observations of incidence of pathogens per category are summarised in Table 2. *Y. enterocolitica*, VTEC and *Campylobacter* have not been listed in Table 2 due to the absence of incidence data of such pathogens in meat products.

2.2. Random-effects meta-analysis

In its simplest form, a meta-analysis can be carried out as a fixed-effects variant to make a *conditional inference* only about the J primary studies included (Hedges & Vevea, 1998). However, most meta-analyses are based on sets of studies that are not exactly identical in their methods and the characteristics of their samples, which may introduce variability (i.e., heterogeneity) among the true effects estimated by the primary studies. One way to model the heterogeneity 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 & Vevea, 1998). It envisions a hypothetical population of studies that comprises studies that have

been conducted, that could have been conducted or that may be conducted in the future. The random-effects model addresses the question: How large is the average true effect in this larger population of studies? In a random effects model, each primary study investigates its own true effect size Θ_j,

$$\theta_j = \Theta_j + \varepsilon_j = \overline{\Theta} + v_j + \varepsilon_j \tag{1}$$

where θ_j is the observed effect size in the primary study j, $\overline{\Theta}_j$ the mean true effect size, and ε_j the error due to sampling variance. The ε_j are assumed to be normally-distributed with mean zero and true variance ξ^2. The term v_j represents the deviation of the true study effect size Θ_j from the mean true effect size. The values of v_j are normally-distributed random effects with mean zero and variance τ^2. Thus, it follows that for a random-effects meta-analysis model, $\theta_j \sim Normal(\Theta, \tau^2 + \xi^2)$. In this approach, two sources of variation are distinguished: sampling variation (ξ^2), and variation between true effect sizes (τ^2). By including this additional component (τ^2), the standard error in the effect size estimates represents random variability at both the subject level and the study level. Notice that the nomenclature θ_j and Θ_j is a general notation that refers to any effect size measure for the observed and the true effect size, respectively. In our particular case, it refers to the logit transformation of the incidence or proportion p_j, which is calculated using the number of successes s_j (positive samples) and total sample size n_j taken from each of the primary studies.

$$\theta_j = logit \ p_j = \log\left(\frac{p_j}{1-p_j}\right) = \log\left(\frac{s_j}{n_j - s_j}\right). \tag{2}$$

The incidence or proportion p_j can be back-transformed as,

$$p_j = \frac{\exp\left(\theta_j\right)}{1 + \exp\left(\theta_j\right)}. \tag{3}$$

Table 2
Number of incidence and observations (s/n) of food-borne pathogens in Portuguese meat products by category extracted from published survey studies.

Pathogen	Intended to be eaten raw	Intended to be eaten cooked	Cured meats
L. monocytogenes	14	11	3
	(1/12), (4/16), (1/15), (1/8), (1/8), (1/7), (1/8), (0/6), (0/10), (2/38), (1/30), (1/44), (1/27), (0/48)	(3/26), (3/24), (1/11), (0/10), (1/11), (0/10), (1/10), (1/10), (0/32), (10/96), (1/9)	(0/66), (0/42), (1/4)
Salmonella	10	13	2
	(1/23), (1/7), (1/15), (0/6), (0/10), (1/38), (1/30), (0/6), (0/6), (0/3)	(0/19), (3/9), (1/1), (1/15), (1/2), (3/10), (0/11), (0/10), (0/10), (1/10), (2/32), (12/96), (0/10)	(1/107), (0/51)
S. aureus	6	7	0
	(1/10), (8/38), (4/30), (2/6), (0/6), (1/3)	(1/10), (3/11), (0/10), (2/10), (0/10), (6/32), (48/96)	

Apart from the values of θ_j from each primary study, the standard error of the effect size must be calculated. The variance of the sampling distribution of the transformed variable is known from statistical theory as

$$\sigma^2\left(\theta_j\right) = \frac{1}{n_j p_j \left(1-p_j\right)}. \tag{4}$$

In order to estimate the mean true effect size from Eq. (1), the observed effect sizes θ_j should be averaged. However, since primary studies usually differ from each other in the reliability of estimating the true effect size (for instance, due to differences in study sizes), a weighted average is preferred with weights w^*_j equal to the precision in estimating the population effect size.

$$w^*_j = \frac{1}{\sigma^2\left(\theta_j\right) + \tau^2}. \tag{5}$$

The variance τ^2 is estimated from the Q-statistic (DerSimonian & Laird, 1986),

$$\hat{\tau}^2 = \frac{Q-(J-1)}{\sum\limits_j w_j - \left(\sum\limits_j w_j^2 / \sum\limits_j w_j^j\right)} \tag{6}$$

where w_j are the weights that would be assigned in a simple fixed-effects model, and Q is a popular statistic used to test the presence of heterogeneity in effect size across primary studies (Cochran, 1954).

$$w_j = \frac{1}{\sigma^2\left(\theta_j\right)} \tag{7}$$

$$Q = \sum\limits_j \frac{\left(\theta_j-\hat{\theta}\right)^2}{\sigma^2\left(\theta_j\right)}. \tag{8}$$

When effect sizes across studies are homogeneous, Q follows a chi-square distribution with $(j-1)$ df. If the hypothesis is rejected, there is evidence that there are additional sources of variability (τ^2) other than within-study sampling error (ξ^2). It is then common practice either to examine moderating variables; to divide the studies in homogeneous groups to perform separate meta-analysis; or to use a random-effects or a multilevel model.

The mean true effect size Θ and its standard error $\sigma(\Theta)$ are now estimated from Eqs. (9) and (10) using instead the corrected weights w^*_j.

$$\hat{\Theta} = \frac{\sum\limits_j w^*_j \theta_j}{\sum\limits_j w^*_j} \tag{9}$$

$$\hat{\sigma}\left(\hat{\Theta}\right) = \frac{1}{\left(\sum\limits_j w^*_j\right)^{0.5}}. \tag{10}$$

To evaluate whether the effect size is larger than zero, often a Wald test assuming normality for the dependent variable is used comparing the estimated weighted average divided by its standard error with a standard normal distribution ($U = \hat{\Theta}/\hat{\sigma}\left(\hat{\Theta}\right)$). The U statistic is compared with a chi-square distribution with one df.

2.3. 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. In a multilevel meta-analysis, as in any other multilevel analyses, one usually starts from the random-effects model (Van den Noortgate & Onghena, 2003). If the between-study variance is shown to be noteworthy, study characteristics or moderators can be added to the model to account for at least part of the heterogeneity in the true effects. This leads to the mixed-effects model given by:

$$\theta_j = \Theta_j + \varepsilon_j = \beta_0 + \sum\limits_{s=1}^{S} \beta_s X_{sj} + v_j + \varepsilon_j \tag{11}$$

with S (X_1 to X_S) study characteristics. This model treats the moderator effects β_s as fixed, and v_j as random effects that distribute normally with a mean 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 S moderators included in the model. The goal of the analysis is then to examine to what extent the moderators influence the size of the average true effect size Θ. The resulting model is thus more general than the ones commonly used in classical meta-analysis. If no predictors are included, the model of Eq. (11) simplifies to the random-effects model (Eq. (1)), or if the variance in true effects is zero, to the fixed-effects model. The use of a regression equation ($\beta_0 + \sum\limits_{s=1}^{S} \beta_s X_{sj}$) for the study characteristics is appealing for several reasons. First, different predictors can easily be investigated together; meaning that even possible inter-correlations can be taken into account, which is not the case when separate meta-analyses are performed to investigate the moderating effects of study characteristics. Secondly, regression is a general approach that can be used for continuous as well as for categorical moderator variables (Van den Noortgate & Onghena, 2003).

2.4. Fitting of models

To estimate the parameters, maximum likelihood estimation (MLE) procedures are most frequently used. In MLE, residuals on both levels (v_j and ε_j of Eq. (1)) are assumed to be independently distributed. To test the fixed parameters of the model (β_s), the Wald test is used like in the traditional approaches, comparing the parameter estimate by the standard error with a standard normal distribution. Apart from the Q statistic, other measures can be computed to facilitate the interpretation of the estimated amount of between-study heterogeneity (τ^2). 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, when the number of studies is small, a lack of significance for τ^2 does not imply that the outcomes are homogeneous. So, they proposed a 25% rule of thumb; this is, if the intra-class variance I^2 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. The I^2 statistic is just a monotonic transformation of τ^2 (see Higgins & Thompson, 2002).

For models including moderators, an omnibus or moderators test (QM test) of all model coefficients is conducted that excludes the intercept. By default, the test statistics of the individual coefficients in the model are based on the normal distribution, while the moderators test is based on a chi-square distribution with S degrees of freedom (S being the number of moderators tested). Finally, after attempting to explain the heterogeneity among studies using the study characteristics,

the QE test can be performed to test the non-explained (residual) variance using the statistic (Raudenbush & Bryk, 1985)

$$QE = \sum_j \frac{\left(\theta_j - \hat{\beta}_0 - \sum_{s=1}^{S} \hat{\beta}_s w_{sj}\right)^2}{\sigma^2\left(\theta_j\right)} \quad (12)$$

which follows a chi-square distribution with J–S–$1°$ of freedom.

As there was considerable dispersion in the number of incidence observations among the meat category − pathogen combinations (Tables 1 and 2), separate meta-analysis studies were conducted on suitable data groups of the combinations presenting more observations. For the meat categories, these were: (i) a meta-analysis on the incidence of *Salmonella* spp. across meat categories; (ii) a meta-analysis on the incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler; and (iii) a meta-analysis on the incidence of *Salmonella* spp., *Y. enterocolitica*, VTEC and *S. aureus* in pork (fresh and minced) for a semi-quantitative risk ranking. For each of the three meta-analyses of pathogens in meats, a random-effects meta-analysis was fitted. The objective of this meta-analysis was to estimate the mean effect size (mean incidence) as well as to assess between-study heterogeneity. Subsequently, multilevel meta-analysis models (Gonzales-Barron et al., 2013) were fitted to each of the three studies using categorical variables, defined as 'meat category' for study (i), and 'pathogen' for studies (ii) and (iii). The general statistical notation of the multilevel model (Eq. (11)) became either $\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j}... + \beta_p X_{pj}) + v_j + \varepsilon_j$ or $\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j}... + \beta_m X_{mj}) + v_j + \varepsilon_j$, where the vector $(\beta_1, \beta_2,...,\beta_p)$ or $(\beta_1, \beta_2,...,\beta_m)$ refers to the shift in effect size coefficients of each pathogen or each meat category for the multilevel model either with pathogens or with meat categories as subgroups, respectively. The coded variable X_p or X_m takes the value of 1 for the pathogen or meat category subgroup. The objective of fitting meta-analyses with a moderator was to assess any statistical difference between subgroups (H_0: $\beta_1 = \beta_2$ ... $= \beta_{p/m} = 0$). Notice that, in this work, the subscript j used in the above equations is general and indistinctly refers to the incidence entry unit for each of the meta-analysis (random-effects and multilevel).

In the case of the meat products, three separate meta-analysis studies were performed on the incidence of each of the three pathogens, *L. monocytogenes*, *Salmonella* spp., and *S. aureus*. For each of the three studies, a random-effects meta-analysis was first adjusted; and subsequently, a multilevel meta-analysis model using 'meat product' as a categorical moderating variable of three levels ($\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j} + \beta_3 X_{3j}) + v_j + \varepsilon_j$). Meta-analysis models were adjusted in R version 2.14.2 (R Development Core Team) using the 'metafor' package (Viechtbauer, 2010), which provides functions for fitting the various models described above as well as meta-analytical graphs (forest plots, funnel plots, etc.).

2.5. Semi-quantitative risk ranking of pathogens in Portuguese raw pork

With the available information of occurrence rates of some pathogens in Portuguese raw pork, a preliminary semi-quantitative risk ranking of *Salmonella* spp., *Y. enterocolitica*, VTEC and *S. aureus* was conducted to identify the top pathogens in terms of public health importance as related to raw pork and potential foodborne infections. The FAO risk assessment grid (FAO, 1998) that qualitatively ranks risk in terms of two variables: severity and likelihood of occurrence, was employed in this study. The categorisation used for the *severity of disease* was the one developed by EFSA (2011a), where the severity of human infection was assessed using lethality among confirmed cases as an indicator, without taking into account whether pork was identified as a source of infection of a case. Using this definition, the severity of consequences is "high" when the human cases are higher than 10/100,000 and case-fatalities are lower than 0.1%. The severity of consequences is "medium" when the human cases are between in 1 and 10/

100,000, and case-fatalities are lower than 0.1%. Lastly, the severity of consequences in pork is low in two cases: when the human cases are lower than 1/100,000 and case-fatalities higher than 0.1%, or when the human cases are lower than 1/100,000 and case-fatalities lower than 0.1% (EFSA, 2011a). According to this severity categorisation, *Salmonella* spp. belongs to high severity, *Y. enterocolitica* to medium severity, while VTEC belongs to low severity. EFSA (2011a) gives *S. aureus* a qualitative category of *unknown* because of the lack of data on frequency and case fatality of confirmed cases.

The second variable is the *likelihood of occurrence* of the pathogen, which ideally should be measured at the point of consumption. However, in practice this type of data is not available, and instead prevalence data at a slaughterhouse or at retail level was used. Occurrence – or even better, concentration – data for the pathogen either at retail level or at another point further up the chain would give some measure of the likelihood of occurrence of the pathogen for the purposes of the risk ranking grid. Thus, in our research, the likelihood of occurrence of a pathogen was replaced by a quantitative estimation of its incidence, obtained by summarising all the occurrences from the primary studies. Box plots of the incidence rates were approached by fitting a beta distribution ($s_T + 1$, $n_T - s_T + 1$) to the incidence data of each of the pathogens (Vose, 2008), where s_T is the total of pooled number of successes (total number of positive samples from all primary studies) and n_T the total sample size.

3. Results and discussion

Although there is a perception that a high incidence of foodborne diseases may have various causes such as inadequate manipulation, preparation and distribution of foods along the food chain, it is also true that quantitative risk assessment leading to efficient prevention demands data availability. Overall, in Portugal, data on microbial contaminants in meats and epidemiology is relatively scarce. For instance, at the level of national compulsory surveillance of infectious diseases, salmonellosis and brucellosis are the only food-related illnesses caused by bacteria subject to notification. In relation to zoonotic agent outbreaks, in 2011, Portugal reported 8 confirmed outbreaks due to toxins of *S. aureus* and *Clostridium* spp. which affected a total of 101 people (EFSA, 2013). Nevertheless, it is not possible to obtain any information about the types of food that mostly contributed to the notified individual cases and outbreaks. The most recent register of the associated foods dates back from 2000, and is found in a report of the European monitoring programme for foodborne disease control (WHO, 2003). According to this study, the foodstuffs mostly associated to the outbreaks in Portugal, in the period 1999–2000, belonged to meat and meat products (21%), followed by baked products (14%) and ready-to-eat meals (22%). Despite the 12-year difference, EFSA (2013) reinforced that meat and meat products – although at EU level – were still the main vehicle of foodborne diseases, responsible for 32% of the strong evidence outbreaks in 2011. In this way, whether eating habits in Portugal have remained basically unchanged in the past decade or the Portuguese situation is well approached by the EU trend, it is imperative to gather as much information as possible on the foodborne pathogens present in Portuguese meats in order to understand the current epidemiological situation, and to allocate resources to prioritise microbial hazards for risk analysis.

The systematic review conducted in this study indicated that currently the information on the level of occurrence of certain pathogens in meats produced in Portugal is sparse. The foodborne pathogens whose presence in meats have not been extensively surveyed are *Campylobacter* (with only 8 published studies identified), *Y. enterocolitica* (7 primary studies), VTEC and *L. monocytogenes* (3 primary studies) and *S. aureus* (2 primary studies). For these pathogens, there is no knowledge (Table 1) on their occurrence or levels in fresh bovine meat, except for one study found for *L. monocytogenes*, and generally no knowledge of their levels in any minced type of meat (i.e., minced beef, minced poultry,
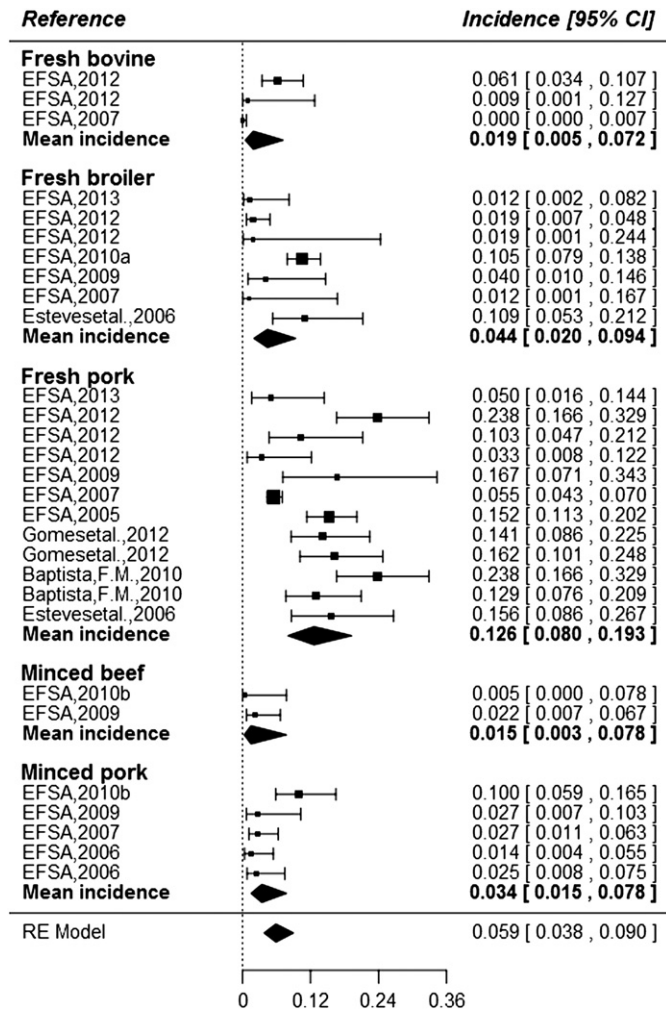
Reference		Incidence [95% CI]
Fresh bovine		
EFSA,2012		0.061 [0.034 , 0.107]
EFSA,2012		0.009 [0.001 , 0.127]
EFSA,2007		0.000 [0.000 , 0.007]
Mean incidence		**0.019 [0.005 , 0.072]**
Fresh broiler		
EFSA,2013		0.012 [0.002 , 0.082]
EFSA,2012		0.019 [0.007 , 0.048]
EFSA,2012		0.019 [0.001 , 0.244]
EFSA,2010a		0.105 [0.079 , 0.138]
EFSA,2009		0.040 [0.010 , 0.146]
EFSA,2007		0.012 [0.001 , 0.167]
Estevesetal.,2006		0.109 [0.053 , 0.212]
Mean incidence		**0.044 [0.020 , 0.094]**
Fresh pork		
EFSA,2013		0.050 [0.016 , 0.144]
EFSA,2012		0.238 [0.166 , 0.329]
EFSA,2012		0.103 [0.047 , 0.212]
EFSA,2012		0.033 [0.008 , 0.122]
EFSA,2009		0.167 [0.071 , 0.343]
EFSA,2007		0.055 [0.043 , 0.070]
EFSA,2005		0.152 [0.113 , 0.202]
Gomesetal.,2012		0.141 [0.086 , 0.225]
Gomesetal.,2012		0.162 [0.101 , 0.248]
Baptista,F.M.,2010		0.238 [0.166 , 0.329]
Baptista,F.M.,2010		0.129 [0.076 , 0.209]
Estevesetal.,2006		0.156 [0.086 , 0.267]
Mean incidence		**0.126 [0.080 , 0.193]**
Minced beef		
EFSA,2010b		0.005 [0.000 , 0.078]
EFSA,2009		0.022 [0.007 , 0.067]
Mean incidence		**0.015 [0.003 , 0.078]**
Minced pork		
EFSA,2010b		0.100 [0.059 , 0.165]
EFSA,2009		0.027 [0.007 , 0.103]
EFSA,2007		0.027 [0.011 , 0.063]
EFSA,2006		0.014 [0.004 , 0.055]
EFSA,2006		0.025 [0.008 , 0.075]
Mean incidence		**0.034 [0.015 , 0.078]**
RE Model		0.059 [0.038 , 0.090]

0   0.12   0.24   0.36

Fig. 1. Forest plot of the multilevel random-effects models of incidence of *Salmonella* spp. in Portuguese meats.

minced pork). Likewise, no single study reporting incidence or numbers of *Y. enterocolitica* in broiler meat or *Campylobacter* in pork have been found. Except for minced broiler meat, *Salmonella* spp. comes out as the only pathogen that has been widely examined in most of the meat categories with 29 primary studies retrieved. This data availability has been prompted by the current national programmes in place to control *Salmonella* in bovine, pig and broiler meat sampled at processing plants and retail. Moreover, the EU mandatory *Salmonella* monitoring programmes at primary production, as well as the enforced food safety criteria for *Salmonella* (Reg. No. 1441/2007) setting limits for specific

food categories, have in turn prompted more scientific research of this pathogen with an integrated food-chain approach.

3.1. Incidence of pathogens in Portuguese meats

The first random-effects meta-analysis indicated that the incidence of *Salmonella* spp. in Portuguese meats is 6% (CI: 4–9%). As displayed in the forest plot (Fig. 1), considerable variability in reported incidence were observed among studies, which was proven by the significant test of heterogeneity (Q = 171; p < 0.001 in Table 3). The between-study variance decreased from $\tau^2 = 1.21$ to 0.58 when meats were grouped in categories by the multilevel model, although there was still some un-explained residual heterogeneity (QE = 130; p < 0.001 in Table 3). The omnibus moderator test (QM) showed that the mean incidence of at least one meat category differed significantly from the others (Table 3). Despite fresh pork corresponds to the category presenting the higher between-study variability (Fig. 1), it is undoubtedly the one with the highest incidence of *Salmonella* spp., diverging significantly from fresh bovine and fresh broiler meat. In contrast with the EU average occurrence for *Salmonella* in fresh pork (0.7%; EFSA, 2013) and minced pork (0.6%; EFSA, 2012), the corresponding occurrence values for Portugal, 12.6% (95% CI: 8.0–19%) and 3.4% (95% CI 1.5–7.8%) are much higher. This finding may be due to the fact that in Portugal there is no bacteriological or serological control programme of pigs on farm that could enable the application of risk management strategies at primary production. Because *Salmonella* is primarily located in the gastrointestinal tract of sub-clinically infected pigs, they introduce the pathogen to the slaughterhouse through their internal organs, skin, faeces, and the cross-contamination of carcasses is basically a matter of redistributing *Salmonella* bacteria from positive pigs during the various slaughter processes. In a risk assessment model, Gonzales Barron et al. (2009) underscored the need to target *Salmonella* contamination at swine production, calculating that on average 77% of the variability in the total contaminated carcasses at the point of evisceration is explained by the contamination from the carrier animals entering the slaughter lines. Thus, although in Portugal there is a national monitoring programme for *Salmonella* in pig meat based on sampling at slaughterhouse and meat cutting plants, the incidence of *Salmonella* in pork is still high, as its control requires rather a systematic approach from farm to fork with specific risk management strategies in place also at farm level. This fact, along with the high consumption of pork in Portugal (43 kg/habitant/year), calls for the contemplation of the implementation of a national *Salmonella* monitoring programme of pig herds. Referring to serovars, the most common ones present in Portuguese fresh pork include Typhimurium (37, 70%), Derby (11, 14%) and Rissen (7, 14%) (as recovered by Baptista, 2010; Gomes-Neves et al., 2012), which belong to the most frequent serovars in pigs and pig meat isolated in the EU (EFSA, 2013).

In relation to the fresh bovine meat and minced beef produced in Portugal, the meta-analysed occurrences of *Salmonella* are 1.9% (95% CI: 0.5–7.2%) and 1.5% (95% CI: 0.3–7.8%), respectively (Fig. 1), which

Table 3
Results of the meta-analysis models for the logit-transformed incidence of *Salmonella* in Portuguese meats. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among meat categories.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.059 (0.038–0.090)[***]	1.21	91.6[*]	–	171[***] (df = 28)
Multilevel					
Fresh bovine	0.019 (0.005–0.072)[a]	0.58[**]	–	49.7[***]	130[***] (df = 24)
Fresh poultry	0.044 (0.020–0.094)[ab]			(df1 = 5, df2 = 24)	
Fresh pork	0.126 (0.080–0.193)[c]				
Minced beef	0.015 (0.003–0.078)[a]				
Minced pork	0.034 (0.015–0.078)[ab]				

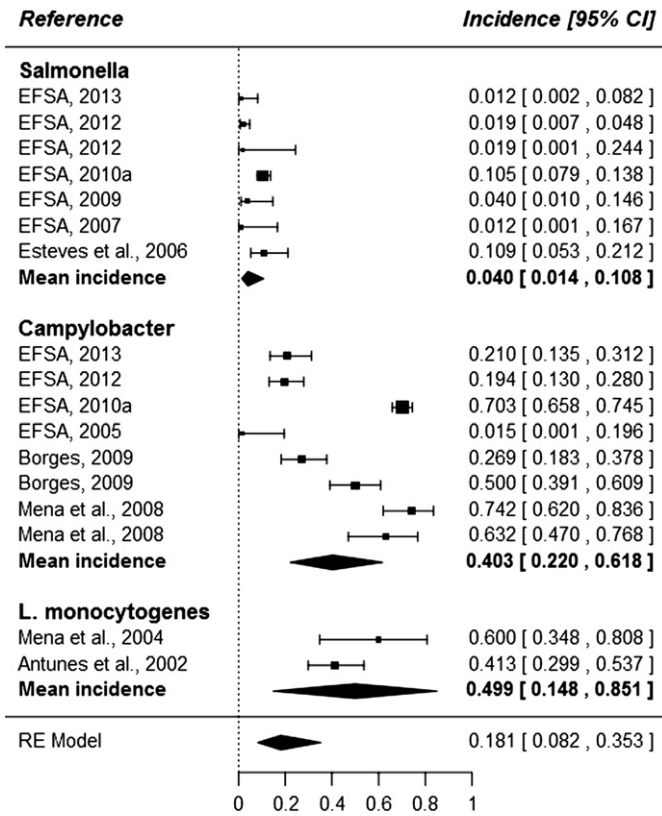Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 [ns] Non-significant.

Fig. 2. Forest plot of the multilevel random-effects models of incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler produced in Portugal.

are values moderately higher than the current EU average (0.2% and 0.4%, respectively; EFSA, 2013). On the contrary, the mean *Salmonella* occurrence in fresh broiler produced in Portugal (4.4%; 95 CI: 2.0–9.4%) appeared to be within the EU level (5.9%). A knowledge gap was found with regard to the *Salmonella* serovars most frequently recovered from Portuguese bovine and broiler meats.

Our study also summarised the occurrence of some pathogens in fresh broiler meat produced in Portugal. The discrepancy among the incidence values of the different pathogens can be visualised in the multilevel meta-analysis forest plot (Fig. 2). As expected, this led to a high between-study heterogeneity ($\tau^2 = 3.22$; Q = 453 with p < 0.001 in Table 4) which still remained significant after categorising the effect sizes by pathogen ($\tau^2 = 1.16$; QE = 178 with p < 0.001). Although a higher between-study variability was observed for *Campylobacter* in comparison with both *Salmonella* and *L. monocytogenes* incidence values (Fig. 2), still the omnibus moderator test (QM) showed that the mean incidence of at least one pathogen differed significantly from the others (Table 4). The fact that *Salmonella* spp. in broiler meat had at least ten times lower incidence than *Campylobacter* and *L. monocytogenes* (Table 4) is believed to be due to the positive impact of the national

Salmonella control programmes at the primary production; this is, in flocks of laying hens, breeding flocks and broiler flocks, with risk reduction targets set in 2007. It is also deemed that the EU compulsory food safety criteria for *Salmonella* (Reg. No. 1441/2007) setting limits for products of meat origin may have contributed to the overall reduction of this pathogen in poultry. The same cannot be said for *Campylobacter* in fresh broiler as its high incidence in Portugal (40.3%; CI: 22.0–61.8%) follows the high EU average trend (31.3%; EFSA, 2013). Since 2005, campylobacteriosis continues to be the most commonly reported foodborne disease in the EU, with broiler meat considered to be the major source of the disease. EFSA (2013) concluded that handling, preparation and consumption of broiler meat may account for 20–30% of human campylobacteriosis in the EU, whilst 50–80% may be attributed to the chicken reservoir as a whole. Moreover, 46% of the *Campylobacter* outbreaks in the EU in 2011, in which the implicated food vehicle was provided, were associated to broiler meat (EFSA, 2013). Nevertheless, it is unfeasible to explore such a link in Portugal since campylobacteriosis cases are neither notified nor its food vehicle investigated. Some research has shown that *Campylobacter jejuni* (15,70%) and *Campylobacter coli* (40,70%) are the two species of highest incidence in Portuguese fresh broiler (as extracted from Borges, 2009; Mena et al., 2008). While there continues to be a lack of epidemiological and risk assessment studies of *Campylobacter* in Portuguese broiler meat, control measures can only be directed to the consumers. Through food labelling and education campaigns, Portuguese consumers should be made aware that adequate cooking will assure safety of meats but serious undercooking or cross contamination from a raw to a cooked product in the kitchen are thought to be major routes of infection.

The association of *L. monocytogenes* as a pathogen of high prevalence in poultry meat, would be considered by many as surprising. However, due to the fact that the high level of mean occurrence of 50% (CI: 14.8–85.1%; Fig. 2) has been obtained from only two available primary studies (Antunes et al., 2002; Mena et al., 2004), at present the main recommendation can be for enabling actions to address the data gap. Djeniyi, Wegener, Jensen, and Bisgaard (1996) indicated that because *L. monocytogenes* is not frequently isolated from chickens, it is likely that the live animals may only contribute little to the total contamination of the abattoir, and that the pathogen may be introduced from dirty transport crates. Even though, poultry has not been associated to a great extent with human listeriosis, variable incidence rates of this pathogen have been found in the past in Denmark (23%; Djeniyi et al., 1996), USA (30%; Cox, Bailey, & Berrang, 1997), Ireland (6–30%; Whyte, McGill, Monahan, & Collins, 2004), Northern Ireland (17%; Soultos, Koidis, & Madden, 2003) and Brazil (28%; Loura, Almeida, & Almeida, 2005). Thus, these and the Portuguese results highlight that the presence of *L. monocytogenes* in Portuguese broiler meat would not be uncommon; and hence, maintaining good hygiene practices in production, processing and the consumer's kitchen cannot be overemphasized.

Within the pork category, occurrence data were available for *Salmonella* spp., *Y. enterocolitica*, *S. aureus* and VTEC (Table 1). The multilevel meta-analysis conducted in this data group also showed that there was significant between-study heterogeneity (Q = 250; p < 0.001 in Table 5).

Table 4

Results of the meta-analysis models for the logit-transformed incidence of pathogens (*Salmonella* spp., *Campylobacter* and *L. monocytogenes*) in Portuguese fresh broiler. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among incidence of pathogens.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.181 (0.082–0.353)[***]	3.22[**]	97.6[*]	–	453[***] (df = 16)
Multilevel					
Salmonella	0.040 (0.014–0.108)[a]	1.16	–	13.8[***] (df1 = 3, df2 = 14)	178[***] (df = 14)
Campylobacter	0.403 (0.220–0.614)[bc]				
L. monocytogenes	0.499 (0.148–0.851)[c]				

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

Table 5
Results of the meta-analysis models for the logit-transformed incidence of pathogens (*Salmonella* spp., *Y. enterocolitica* and VTEC) in Portuguese fresh and minced pork. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among incidence of pathogens.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.085 (0.053–0.134)[***]	1.54[**]	92.6	–	250[***] (df = 28)
Multilevel					
Salmonella	0.086 (0.048–0.152)[a]	1.45	–	1.5[*]	173[***]
Y. enterocolitica	0.068 (0.022–0.193)[a]			(df1 = 3, df2 = 25)	(df = 25)
VTEC	0.056 (0.012–0.221)[a]				
S. aureus	0.500 (0.073–0.927)[b]				

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

Furthermore, while the mean incidence rates of both *Y. enterocolitica* and VTEC in pork were numerically lower than the *Salmonella* mean incidence, yet statistical differences among these occurrence rates could not be revealed (Table 5). Pig meat and pork products are considered to be the most important source of pathogenic *Y. enterocolitica* infections in humans (EFSA, 2012, 2013; Simonová, Vázlerová, & Steinhauserová, 2007). The meta-analysed mean value of this pathogen in Portuguese pork (6.8%; 95% CI: 2.2–19.3%) was moderately higher than the overall incidence rate of 2.4% (30 out of 1146 samples) reported for four EU countries in 2011 (EFSA, 2013). It is the ability of this organism to grow at 4 °C which makes refrigerated pork preparations with a relatively long shelf-life a probable source of infection (EFSA, 2005). Although the occurrence of VTEC in Portuguese pork was meta-analysed from only four primary studies, like *Y. enterocolitica*, VTEC's mean occurrence (5.6%; 95% CI: 1.2–22.2% in Table 5) turned out to be higher than the EU average reported over the years 2007–2010 (EFSA, 2012). Many of the investigations reported to EFSA in those years did not yield any positive findings, except for six countries which found VTEC incidence in fresh pig meat at very low levels (0.1–2%). VTEC O157:H7 was detected in three of these national surveys, and the highest proportion of positive samples was reported by Spain (1.2%; EFSA, 2012). On the other hand, although bovine meat is believed to be a major source of foodborne VTEC infections for humans, no microbiological survey of VTEC in bovine meat has been identified in Portugal. This is an issue that merits attention as VTEC infections in humans have been following an increasing trend since 2006 in a number of European countries such as the Netherlands, Austria, Denmark, Finland, France and Luxembourg (EFSA, 2012).

3.2. Risk ranking of pathogens in Portuguese raw pork

A representation of the semi-quantitative risk ranking is shown in Fig. 3, where the confidence intervals of the box plots do not depict the uncertainty around the mean incidence (which is described by the confidence intervals of the meta-analysed mean incidences of Table 5), but instead the total uncertainty around the true incidence in a Bayesian framework. For this reason, the confidence intervals of the box plots (Fig. 3) are larger than those obtained by meta-analysis in Table 5. The pathogens were then categorised regarding their incidence or frequency of occurrence as per the criterion suggested in EFSA (2011a). This criterion establishes that the frequency of detection can be high, medium or low if the prevalence is higher than 5%, between 0.1 and 5% and lower than 0.1%, respectively. In our case, all four pathogens were assigned to the "high" frequency of detection category as at least 50% quartile of their box plot were above the cut-off criterion of 5%.

Bringing together the two variables, severity of disease and likelihood of occurrence, using the risk assessment grid from FAO (1998), it was found that the significance of *Salmonella* spp. in the fresh pork produced in Portugal is *critical* while the significance of *Y. enterocolitica* and VTEC is *major* and *minor*, respectively. In the case of *S. aureus*, there was a lack of data on intoxication severity, and also scarce knowledge on the likelihood of occurrence in Portugal (as only one primary study was retrieved). While such study (Esteves, Saraiva, et al., 2006) reported a high mean incidence of 50% in pork cuts, there is no knowledge on isolated strains capable of toxin formation. It is known that pork-derived products may remain a potential source of meticillin-resistant *S. aureus* (MRSA), with CC398 being the MRSA lineage most commonly associated to intensively-reared food-producing animals. However, so far there is no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398 (Smith et al., 2011). Furthermore, as the main risk factors of *S. aureus* include cross contamination and growth at processing, retail and domestic levels, EFSA (2011a) ranked the significance of *S. aureus* in chilled pig carcasses as low or minor risk. In our preliminary assessment, the microbiological hazards ranking in descending order were then: *Salmonella* spp., *Y. enterocolitica*, VTEC, while *S. aureus* was assigned to an unknown risk category (Fig. 3). A totally different outcome was found when using the ICMSF (2002) criteria for the severity characterisation of pathogens. In such a case, all three pathogens VTEC, *Salmonella* and *Y. enterocolitica* represented major risks. Thus, it is imperative that national resources and efforts be allocated to the implementation of a system for control and prevention to reduce, in the first place, the current high levels of *Salmonella* and *Y. enterocolitica* in the Portuguese pig herds and at processing level.

3.3. Incidence of pathogens in Portuguese meat products

L. monocytogenes has not only been detected in a variety of raw meats, but also in food contaminated post-processing such as cheeses and RTE meats, both of which have been implicated internationally in
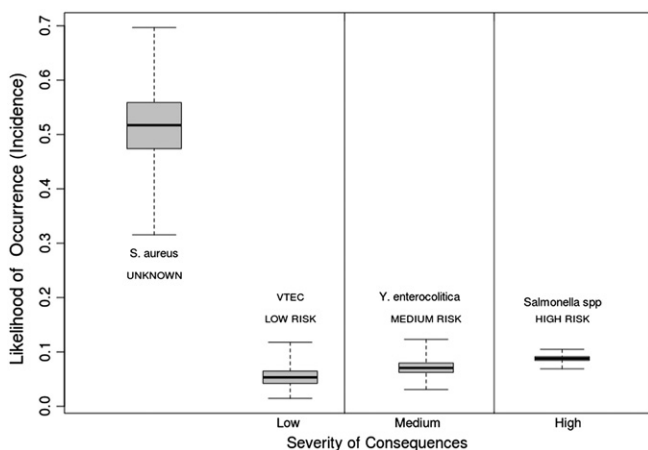

Fig. 3. Semi-quantitative risk ranking of pathogens associated with Portuguese fresh pork. The categorisation of severity of consequences was taken from EFSA (2011a).

Table 6
Results of the three meta-analysis models for the logit-transformed incidence of pathogens (*L. monocytogenes*, *Salmonella* spp., *S. aureus*) in Portuguese meat products. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among meat product categories.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
L. monocytogenes					
Fixed-effects	0.088 (0.065–0.118)***	0	0	–	22.2[ns] (df = 27)
Multilevel		–	–	0.87[ns] (df1 = 2, df2 = 25)	20.8[ns] (df = 25)
To be cooked	0.098 (0.066–0.144)[a]				
To be eaten raw	0.083 (0.051–0.131)[a]				
Cured	0.042 (0.010–0.162)[a]				
Salmonella spp.					
Fixed-effects	0.097 (0.070–0.134)***	0	0	–	32.5[ns] (df = 24)
Multilevel		–	–	8.13*** (df1 = 2, df2 = 22)	18.7[ns] (df = 22)
To be cooked	0.134 (0.092–0.190)[c]				
To be eaten raw	0.057 (0.028–0.113)[b]				
Cured	0.009 (0.002–0.045)[a]				
S. aureus					
Random-effects	0.226 (0.154–0.318)***	0.23	39.5**	–	33.5*** (df = 12)
Multilevel		0.24	–	13.5*** (df1 = 2, df2 = 11)	24.0* (df = 11)
To be cooked	0.258 (0.157–0.393)[b]				
To be eaten raw	0.184 (0.097–0.319)[a]				

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

outbreaks. In contrast to the meta-analysis in Portuguese meats, which presented a high variability among studies (see spread of measured outcomes in Fig. 1), the meta-analysis of *L. monocytogenes* in meat products did not have a significant between-study variability (Q = 22, p > 0.05; Table 6), as can be also visualised in the respective forest plot (Fig. 4). The reduced spread in the measured incidences (Fig. 4) across the great variety of meat products, may be due to the fact that *L. monocytogenes* enters the chain mainly through the raw meat; and subsequently its survival is affected by common ingredients and processing steps (i.e., salt, spices, thermal processing of some ingredients/meats, fermentation, smoking and drying), in a way that such combined effects may be comparable among meat products. Thus, the random-effects model of Eq. (1), with a between-study variability of $\tau^2 = 0$, reduced to a fixed-effects model. The twenty-six meat products were grouped in three categories: to be cooked, to be eaten raw and cured meats, in order to conduct the multilevel fixed-effects meta-analysis. The first two sub-groups contained mainly traditional meat products, many of them bearing quality labels, and the last sub-group was comprised of products elaborated with curing salts and produced to a larger scale. Although discrepancies among the mean incidence values of *L. monocytogenes* in the three categories were not large enough to cause statistical significance (i.e., the number of primary studies for the cured meats were few in comparison to the other two categories, which brought about a greater uncertainty around its mean estimate), still the mean incidence for the cured meats was numerically lower than the others (Table 6). This lower rate (4.2%; 95% CI: 1.0–16.2%) may be due to the fact that these products contain nitrites and undergo some heat treatment; yet it can be said that this value is within the order of magnitude of the overall EU incidence of 2.4% for RTE products of meat origin except fermented sausages reported in 2010 (EFSA, 2012). It is worthwhile to mention that this EU mean incidence is taken within the context of compliance to the microbiological criterion of *L. monocytogenes* in RTE foods, expressed as a percentage of single samples and not of batch units; and in both, Portuguese studies and EFSA results, the stipulated sample weight of 25 g was used. The mean incidence of *L. monocytogenes* in Portuguese meat products intended to be eaten cooked (9.8%; 95% CI: 6.6–14.4%) was high and not different (p > 0.05) from the mean occurrence in fermented sausages (8.3%; 95% CI: 5.1–13.1%; Table 6). Although the doses (concentration) of *L. monocytogenes* in these products may as well be low, this is an outcome that merits further investigation since such fermented

meats are of high consumption and commonly eaten in Portugal without any further cooking that would reduce the risk of contracting the pathogenic agent by the consumers. Although it may not be directly comparable, at EU level the non-compliance of *L. monocytogenes* in fermented sausages sampled at processing plants was of 1.0% in 2011
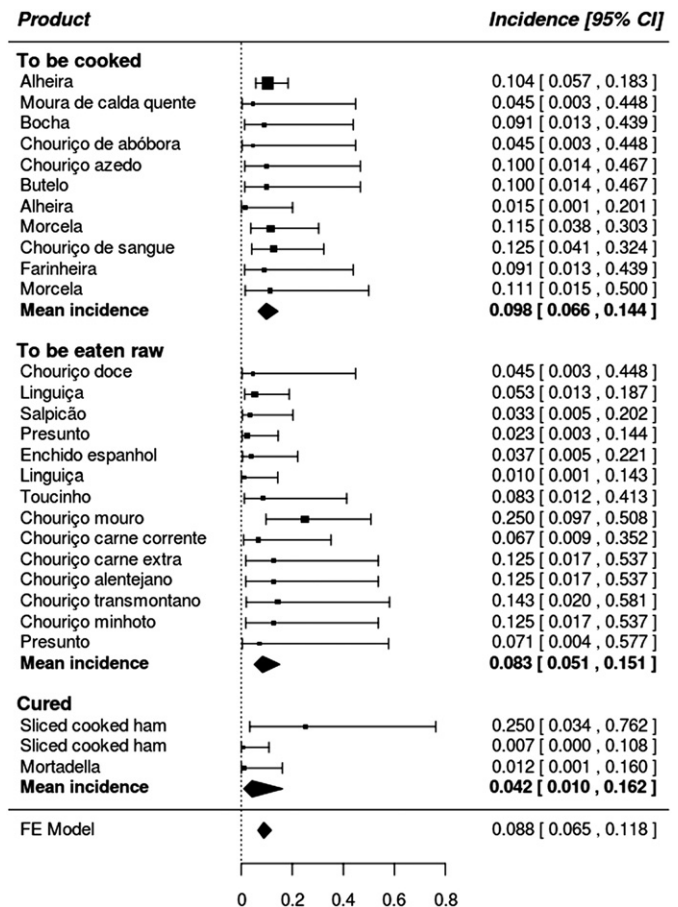

Fig. 4. Forest plot of the multilevel random-effects model of incidence of *L. monocytogenes* in Portuguese meat products.
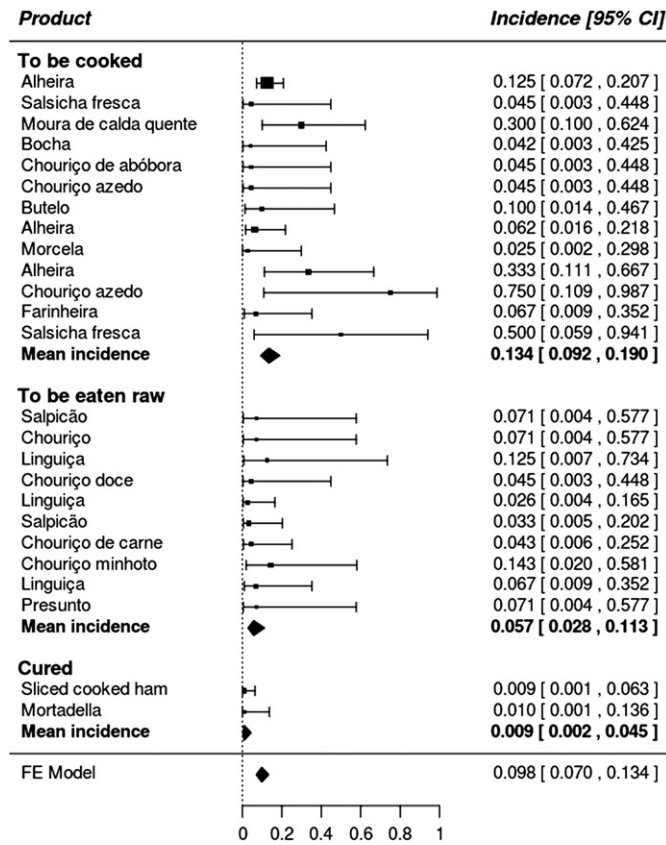
Fig. 5. Forest plot of the multilevel random-effects model of incidence of *Salmonella* spp. in Portuguese meat products.

(EFSA, 2013), which is a level fairly below the meta-analysed estimate for Portugal. These results underscore the necessity, firstly, to understand the risk factors in the processing of traditional meat products contributing to the pathogen's high prevalence; and secondly, to assess suitable risk-based control measures along production.

The meta-analysis of *Salmonella* spp. in meat products, like the meta-analysis of *L. monocytogenes* referred above, also led to a fixed-effects solution, as there was no significant variability among products
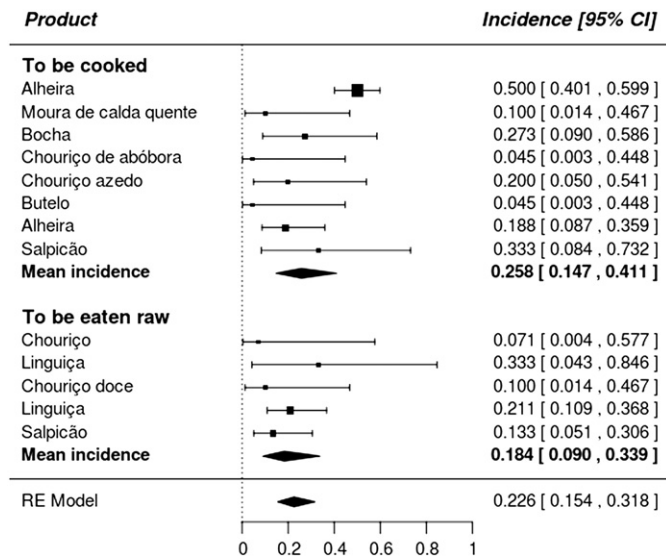

Fig. 6. Forest plot of the multilevel random-effects model of incidence of *S. aureus* in Portuguese meat products.

(Q = 32, p > 0.05; and hence $\tau^2 = 0$ in Table 6). Again, the homogeneity in the measured incidences of *Salmonella* spp., despite the great variety of meat products considered, (Fig. 5) was noteworthy. The fact, that the combined effects of ingredients and processing on the viability of *Salmonella* along production may be comparable among the different meat products, could explain the absence of between-study heterogeneity in *Salmonella* occurrence. The overall mean incidence of *Salmonella* in Portuguese meat products was high (9.7%; 95% CI: 7.0–13.4%) and did not differ (p > 0.05) from the mean incidence of *L. monocytogenes* (8.8%; 95% CI: 6.5–11.8% in Table 6). In the case of cured meats, the incidence rate of *Salmonella* spp. (0.9%; 95% CI: 0.2–4.5%) was considerably lower than the incidence of *L. monocytogenes* (4.2%; 95% CI: 1.0–16.2%), which may be due to the lower resistance of *Salmonella* spp. to curing agents, heat treatment and cold ripening (Hwang et al., 2009). Among the three meat product categories compared by the multilevel meta-analysis (Table 6), there were significant differences in mean *Salmonella* occurrence, with the traditional meat products intended to be eaten cooked having the highest incidence (13.4%; 95% CI: 9.2–19.0%). This level of non-compliance to the *Salmonella* EU microbiological criterion (in single samples unit) was higher than the EU levels reported in 2011 for the following related categories (EFSA, 2013): poultry meat preparations intended to be eaten cooked (6.8%), meat preparations from other species than poultry intended to be eaten cooked (1.1%), and meat products from poultry intended to be eaten cooked (1.1%). Given such a high incidence of *Salmonella* in the Portuguese meat products to be cooked, and the severity of this pathogen, actions should be enabled to lower *Salmonella* prevalence in the pork industry as well as to instruct consumers of the importance of fully cooking the raw traditional meat products and avoiding cross contamination. On the other hand, the meat products intended to be eaten raw presented a lower *Salmonella* incidence of 5.7% (95% CI: 2.8–11.3% in Table 6), which, nonetheless, was still numerically higher than the EU average for the meat preparations intended to be eaten raw in 2011 (from 0 to 1.4%; EFSA, 2013). It is worthy to mention that since 2008 the highest level of non-compliance with the EU *Salmonella* microbiological criteria generally occurred in food of meat origin with minced meat and meat preparations from poultry intended to be eaten cooked having the highest level of non-compliance.

A different scenario was noticed for the meta-analysis conducted on the presence of *S. aureus* in Portuguese meat products (Fig. 6). There, a significant heterogeneity among products was observed (Q = 33, p < 0.05) with an I^2 suggesting that ~40% of the total variability in measured occurrences can be attributed to the variability product to product. Even after meat products were categorised in 'to be eaten raw' and 'to be eaten cooked', there was some remaining between-product variability (residual QE = 24, p < 0.05) in *S. aureus* incidence. Unlike *L. monocytogenes* and *Salmonella* spp., whose incidence rates were rather homogeneous among the meat products, the heterogeneity in the occurrence of *S. aureus* can only reveal the variability in hygiene among the different meat products during manufacturing. On average, 22.6% (95% CI: 15.4–31.8%; Table 6) of the Portuguese meat product samples are expected to be contaminated with this pathogen. The high occurrence of *S. aureus* in these meat products is an indicator of hygiene deficiency during processing. For instance, for Alheira (a raw meat product, first in Fig. 6), a product whose processing implies extensive manipulation, the incidence of *S. aureus* was the highest (50%). In the EU, staphylococcal enterotoxins were the causative agent of 6% (435 outbreaks) of all outbreaks reported in 2011; and along with other toxins produced by *Clostridium* and *Bacillus*, they ranked second as responsible agents of all the foodborne outbreaks in the EU, only after salmonellosis. In Portugal, and for the same year, 6 confirmed outbreaks due to staphylococcal toxins occurred, involving a total of 90 human cases. It is also known that the largest proportion of strong-evidence outbreaks caused by staphylococcal toxins is attributed to mixed food including meats (40%) (EFSA, 2013). Giletto and Fyffe (1998) indicated that the food vehicles most frequently involved in intoxication by *S. aureus*, are cooked
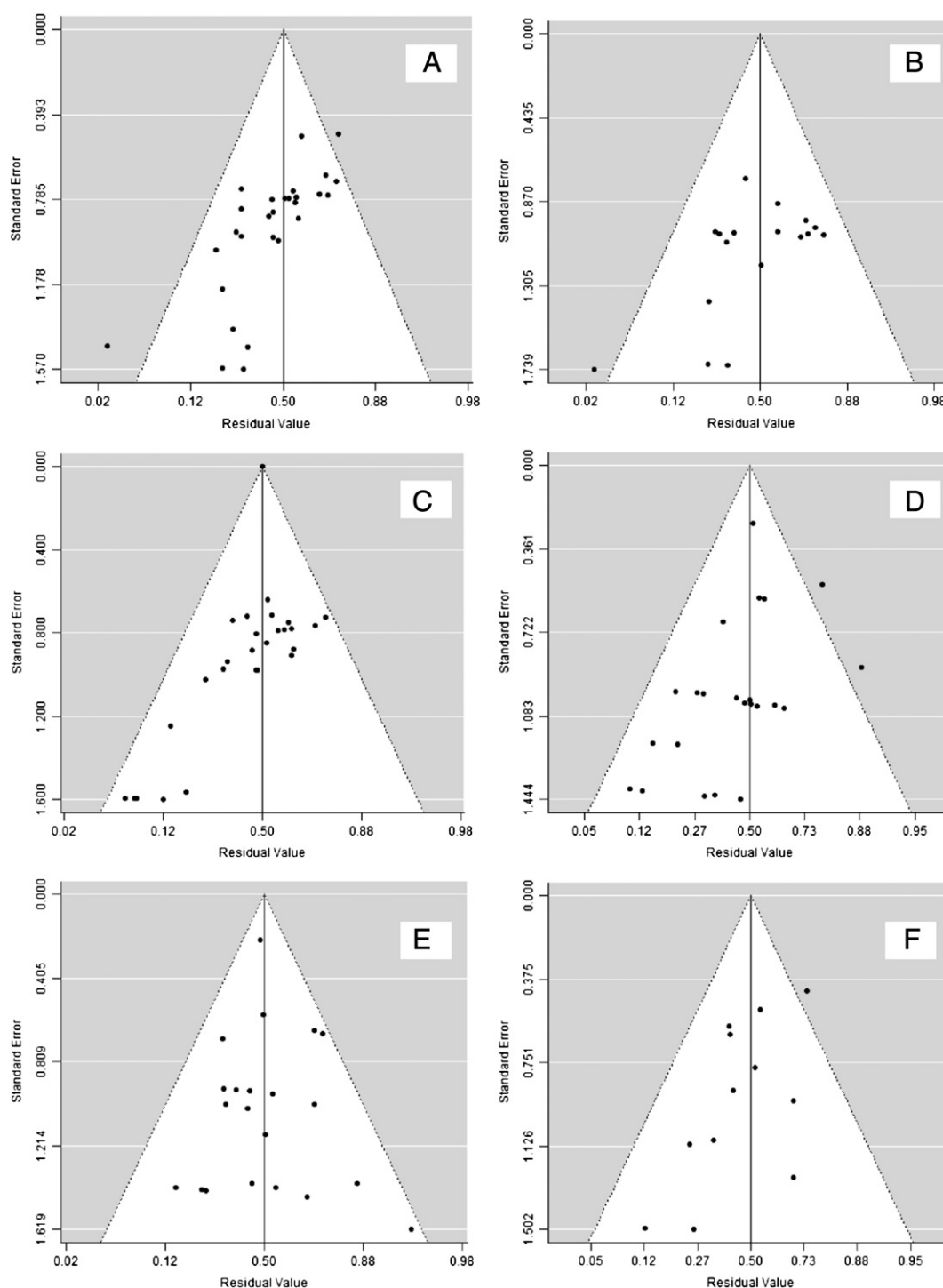
Fig. 7. Funnel plots of the incidence of *Salmonella* spp. in Portuguese fresh meat (A); the incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler (B); the incidence of *Salmonella*, VTEC, *Y. enterocolitica* and *S. aureus* in pork (C); and the incidence of *L. monocytogenes* (D), *Salmonella* spp. (E) and *S. aureus* (F) in Portuguese meat products.

refrigerated meats and meat products, such as turkey meat, roasted meat, ham and fermented meat products as well as bakery products containing cream. Thus, the outcome of this meta-analysis, which points towards a very high incidence of *S. aureus* in both meat products to be cooked (25.8%; 95% CI: 15.7–39.3%) and meat products to be eaten raw (18.4%; 95% CI: 9.7–31.9%) may support the current epidemiological situation of foodborne outbreaks in Portugal; at least to some extent as also other foods can be vehicles of staphylococcal enterotoxins. Furthermore, considering that the background microflora might limit the growth of *S. aureus* and therefore, the toxin production, further research should be carried out from a dose-response perspective.

As part of this study, publication bias was also investigated for each of the six multilevel meta-analyses conducted. Firstly, this was done by the construction of a funnel plot, which relates the mean incidence value from each primary study (or its residual after removing the covariate effects in the case of a multilevel meta-analysis) with its respective standard error as a measure of the level of confidence in the results of such primary study (for further details on the funnel plot, refer to Whitehead, 2002). There was a general tendency (Fig. 7) in having very few or a total absence of publications reporting high incidence values (higher residuals in the x-axis of the funnel plot) from small sample sizes (higher standard errors). This can be verified by the blank right

bottom area in most funnel plots (Fig. 7). Nevertheless, some caution should be taken in identifying this phenomenon directly as a proof of publication bias, since it is quite common that a small sample size will fail to detect any pathogen if this is present in lower concentrations. Said otherwise, it is likely that a sample size consisting of five sample units will not have the statistical power to detect *Salmonella* spp. if the true prevalence of this pathogen in the food under question is very low. For this reason, in microbiological surveys of absence/presence of pathogens in foods, in order to accurately estimate the pathogen's prevalence, a large sample size is commonly required when its concentration in food is known to be low. This is the same phenomenon which produces the high number of zero counts normally observed in microbial data (Gonzales-Barron & Butler, 2011). Thus, some caution should be taken when interpreting the funnel plots of incidence data as the blank right bottom area is not necessarily a proof of publication bias. Another approach to test publication bias is to investigate the effect of the study size directly by including the total sample size of a study as explanatory variable in a multilevel meta-analysis. Results of these multilevel meta-analyses (not shown) suggested that there was no significant effect of sample size on the observed incidences. Hence, as the presence of unpublished small-size studies reporting high prevalence is very unlikely, it is highly probable that the effect size outcomes (mean incidences) presented in these meta-analysis are not affected by publication bias.

4. Conclusion

The systematic review conducted in this research allowed to recognise the sparseness of knowledge on the incidence of pathogens in meats and meat products produced in Portugal. This meta-analysis study provided the first pooled incidence estimates for pathogens in specific meat categories, which are more robust and reliable than single study estimates. For the meta-analyses conducted on Portuguese meats categorised by origin, a greater number of incidence observations from primary studies were sourced for *Salmonella* in the different meats and *Campylobacter* in broiler meat than in all the other pathogen-meat combinations. The meta-analysed mean occurrence rate of *Campylobacter* in broiler meat (40%) was found to be nearly ten times higher than that of *Salmonella* (4.4%), although both levels were well within EU ranges. The lower incidence of *Salmonella* in broiler meat may be explained by the positive impact of the national control programmes in flocks of laying hens, breeding flocks and broiler flocks in place from 2008. In other fresh meat categories (i.e., bovine meat, pork, minced beef and minced pork), the *Salmonella* mean incidence values for Portugal were in all cases slightly to moderately higher than EU averages, being lowest in fresh bovine (1.9%) and highest for pig meat (12.6%). Furthermore, the semi-quantitative risk ranking in Portuguese pork assigned to *Salmonella* spp. and *Y. enterocolitica* (6.8% occurrence) the risk categories of critical and major, respectively. Considering that, in Portugal the meat of pig origin has the highest consumption per capita, it is essential that a comprehensive pork carcass safety assurance be implemented. The current monitoring programme, consisting in testing for *Salmonella* in pig carcasses at the slaughterhouses, per se will not lead to any risk mitigation unless reduction targets are set to be achieved for *Salmonella* and *Y. enterocolitica* in/on chilled pig carcasses. It is equally necessary that, earlier in the food chain, risk management strategies such as differentiation of both pig batches and abattoirs as well as on-farm strategies such as categorisation of pig herds and herd health programmes be implemented in Portugal. It was surprising to find few primary studies reporting high occurrences of *L. monocytogenes* in Portuguese broiler (50%) and *S. aureus* in pork (50%), which are issues of concern that should be addressed by further research. This meta-analysis also highlighted the areas where there are gaps of knowledge such as the *Salmonella* serovars in bovine and broiler meats, the occurrence of VTEC in fresh bovine meat, *Y. enterocolitica* in fresh bovine and broiler meats, *Campylobacter* in pork, and in general the lack of information on the presence of toxin-producing pathogens in raw meats.

In the case of the Portuguese meat products, incidence data was only available for *L. monocytogenes*, *Salmonella* spp. and *S. aureus*. Data gaps were recognised for toxin-producing microorganisms such as *Clostridium* spp. and *Bacillus cereus*. The overall incidence of *S. aureus* (22.6%) in the Portuguese meat products was significantly higher than those of *L. monocytogenes* (8.8%) and *Salmonella* (9.7%), which indicates a breakdown in processing hygiene. Bringing together the great variety of meat products produced in Portugal, it was found that the meat products 'intended to be eaten cooked' had mean incidences generally higher than the meat products 'to be eaten raw' for the three pathogens assessed. Nevertheless, since the mean occurrence rates or non-compliance rates to the EU microbiological criteria for both *L. monocytogenes* (9.8% in products to be cooked and 8.3% in products to be eaten raw) and *Salmonella* (13.4% in products to be cooked and 5.7% in products to be eaten raw) were considerably higher than EU levels for RTE products in comparable categories, it is essential that Portuguese food safety agencies take actions for the maintenance of good hygiene practices in the production and processing of traditional meat products. Putting all information together, this meta-analysis work revealed a clearer picture of the state of knowledge on the incidence of the most important foodborne pathogens in Portuguese-produced meats and meat products. It may also assist national food safety authorities and policy makers in the prioritisation of microbiological hazards in the specific meat type categories, and can equally aid researchers to provide direction for future investigation.

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