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

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ABSTRACT

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.

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