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Quantifying *Listeria monocytogenes* prevalence and concentration in minced pork meat and estimating performance of three culture media from presence/absence microbiological testing using a deterministic and stochastic approach

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ABSTRACT

Listeria monocytogenes poses a serious threat to public health, and the majority of cases of human listeriosis are associated with contaminated food. Reliable microbiological testing is needed for effective pathogen control by food industry and competent authorities. The aims of this work were to estimate the prevalence and concentration of *L. monocytogenes* in minced pork meat by the application of a Bayesian modeling approach, and also to determine the performance of three culture media commonly used for detecting *L. monocytogenes* in foods from a deterministic and stochastic perspective. Samples ($n = 100$) collected from local markets were tested for *L. monocytogenes* using in parallel the PALCAM, ALOA and RAPID'L.mono selective media according to ISO 11290-1:1996 and 11290-2:1998 methods. Presence of the pathogen was confirmed by conducting biochemical and molecular tests. Independent experiments ($n = 10$) for model validation purposes were performed. Performance attributes were calculated from the presence–absence microbiological test results by combining the results obtained from the culture media and confirmative tests. Dirichlet distribution, the multivariate expression of a Beta distribution, was used to analyze the performance data from a stochastic perspective. No *L. monocytogenes* was enumerated by direct-plating (<10 CFU/g), though the pathogen was detected in 22% of the samples. *L. monocytogenes* concentration was estimated at 14–17 CFU/kg. Validation showed good agreement between observed and predicted prevalence (error = −2.17%). The results showed that all media were best at ruling in *L. monocytogenes* presence than ruling it out. Sensitivity and specificity varied depending on the culture-dependent method. None of the culture media was perfect in detecting *L. monocytogenes* in minced pork meat alone. The use of at least two culture media in parallel enhanced the efficiency of *L. monocytogenes* detection. Bayesian modeling may reduce the time needed to draw conclusions regarding *L. monocytogenes* presence and the uncertainty of the results obtained. Furthermore, the problem of observing zero counts may be overcome by applying Bayesian analysis, making the determination of a test performance feasible.

1. Introduction

Minced meat supports the growth of a wide variety of microorganisms including *Listeria monocytogenes*, the causative agent of listeriosis (Lianou and Sofos, 2007; Sofos et al., 1995). Despite the low incidence of the disease in humans, listeriosis is characterized by high hospitalization and case fatality rates (i.e. 20–30%), especially among Young, Old, Pregnant and Immune-compromised (YOPI) people (Mead et al., 1999). During the past three decades, the recorded cases of *L. monocytogenes* infections have been grown significantly due to several outbreaks of foodborne listeriosis. The contamination levels in foods associated with *L. monocytogenes* infection were between 10^2 and 10^6 CFU/g or CFU/ml in the majority of cases (Dawson et al., 2006). The initial population of the pathogen in food products, which in most cases is below the detection limit (10 CFU/g based on ISO 11290-2:1998) (ISO, 1998), plays a critical role since the microorganism is able to grow at low temperatures due to its psychrotrophic nature

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(NACMCF, 2005). Therefore, the existence of a reliable method for the detection of *L. monocytogenes* in foods is a prerequisite.

Predictive or quantitative microbiology describes microbial responses to a given set of conditions (e.g. temperature, pH and α_w) by mathematical equations (models). The models can be used to make predictions regarding pathogenic or spoilage microorganisms' behavior under specified environmental conditions (McDonald and Sun, 1999; Vadasz and Vadasz, 2008). The majority of published models adopt a deterministic approach to the prediction of microorganisms' behavior, without taking account of possible variability and/or uncertainty in the output data, thus limiting their usefulness, for risk assessment and other purposes (Nauta, 2002; Pouillot and Lubran, 2011). Consequently, a more stochastic approach is needed; particularly as it is well known that routine culture-dependent methods are imperfect diagnostic tests with sensitivities and specificities less than 100% (Habib et al., 2008; Rosenquist et al., 2007). Estimation of pathogen prevalence from survey or screening test data can be erroneous. However, the true rather than the apparent prevalence can easily be calculated if the sensitivity and specificity of the test(s) used in surveys or for screening are known (Thrusfield, 2007). In this way, undesirable under- or overestimation of prevalence can be avoided, thus enhancing understanding of the extent to which pathogens are in control at each stage in a food production chain. Recently, Bayesian methods have been introduced for predicting microbial growth parameters, with improvement of the accuracies of probabilistic models (Crepet et al., 2009; Delignette-Muller et al., 2006; Jaloustre et al., 2011; Pouillot et al., 2003). Bayesian analysis combines prior knowledge, described by probability distributions, with the available data to produce updated posterior distributions (Lesaffre et al., 2007).

The International Organization for Standardization (ISO) has published in 1996 and 1998 two protocols for the detection and enumeration of *L. monocytogenes* (ISO, 1996, 1998). In these protocols, OXFORD and PALCAM agars have been proposed as isolation media. These media, however, do not allow differentiation of the pathogen from other *Listeria* species and alternatively the use of chromogenic media such as ALOA, RAPID'L.mono or OCLA has been suggested (Becker et al., 2006; Reissbrodt, 2004). The published standards are currently under revision but the use of such alternative plating methods requires testing of their performance in order to prove their suitability. Becker et al. (2006) tested and compared the performance of ALOA and RAPID'L.mono with that of OXFORD and PALCAM plating media using naturally contaminated ready-to-eat foods such as smoked salmon, raw and cooked sausages and ready-to-eat salads taken from retail outlets. Nevertheless, the performance was expressed only as a percentage of confirmed positive samples by any method. The culture media currently used to detect *L. monocytogenes* in foods are not perfect meaning that their sensitivity and specificity are lower than ideal (i.e. one), whilst relevant values may vary depending on the medium used. As a result, a variation in detection of the pathogen could occur due to the culture-dependent method followed during microbiological analysis of foods, even when the same sample is analyzed. Vlaemynck et al. (2000) found increased prevalence of *L. monocytogenes* in naturally contaminated dairy and meat products by 4.3% when ALOA was used instead of PALCAM or OXFORD. International trials performed to compare chromogenic media for *L. monocytogenes* detection with PALCAM or OXFORD revealed that ALOA and RAPID'L.mono performed better or at least equal to the two aforementioned media (Reissbrodt, 2004). Therefore, the true prevalence of the pathogen cannot be accurately determined based on a test alone. When imperfect tests are used with unknown values regarding sensitivity and specificity then the number of parameters that should be estimated is higher (apparent prevalence, sensitivity and specificity of the test) than the number of equations [true prevalence = (apparent prevalence + specificity − 1)/(sensitivity + specificity − 1)] (Berkvens et al., 2006; Lesaffre et al., 2007). The experimental data provide information only for the apparent prevalence (i.e. number of positive samples according to the test divided by the total number of samples analyzed) but no information for the other two. Hence, sensitivity and specificity should be obtained from other sources such as other experimental data, other studies, expert opinion or even use of special type of distributions when no prior knowledge is available (Lesaffre et al., 2007; Vose, 2008). This can be achieved through Bayesian analysis. Furthermore, the use of multi-testing, which is the subjection of each sample to different diagnostic tests, in conjunction with Bayesian inference allows better estimation of true prevalence for *L. monocytogenes*. The first leads to introduction of extra variables, while the second combines prior knowledge with experimental data and thus estimates are obtained for both true prevalence and diagnostic test characteristics. In Bayesian analysis, probability distributions assigned to the prior knowledge, are combined with experimental data to provide updated posterior probability distributions (Lesaffre et al., 2007). The difference between deterministic and Bayesian approach regarding determination of true prevalence, sensitivity and specificity is the fact that sensitivity and specificity are also variables in the Bayesian, whereas in the deterministic approach are fixed parameters with apparent prevalence being the only variable (Berkvens et al., 2006; Lesaffre et al., 2007). Consequently, the major advantage of Bayesian analysis over deterministic is the better estimation of true prevalence and diagnostic test characteristics by combining results from imperfect tests, which otherwise would remained undefined (Pouillot et al., 2002).

Therefore, the aims of this study were 1) to accurately estimate the prevalence and concentration of *L. monocytogenes* in minced pork meat from presence–absence microbiological data by the application of a Bayesian modeling approach, 2) to thoroughly determine the performance of three culture media commonly used for detecting *L. monocytogenes* following a deterministic approach and 3) to determine the diagnostic accuracy measures such as classification probabilities, predictive values, likelihood ratios and area under receiving operating characteristic curve of three different culture-dependent methods commonly used for detection of the foodborne pathogen *L. monocytogenes* from a stochastic perspective, handling also the uncertainty of these estimations by means of Bayesian analysis and parallel testing. Stochastic approach has the following advantages over deterministic: a) better handling of zero counts observed for some of the parameters, b) better handling of parameters, uncertainty and c) better estimates for true prevalence and diagnostic test characteristics. To this end, PALCAM, ALOA and RAPID'L.mono were tested and compared using naturally contaminated minced pork meat purchased from local butchers' shops and supermarkets.

2. Materials and methods

2.1. Collection of minced pork meat samples

Samples ($n = 100$) of naturally contaminated fresh minced pork meat (weight of each sample equal to 500 g), prepared upon request from aerobically stored meat cuts kept at refrigerating temperatures, were purchased from 100 different retail outlets (local butchers' shops and supermarkets) within the metropolitan area of Athens, Greece, during a survey between May 2009 and March 2010. Samples were transported to the laboratory, under refrigeration in polystyrene boxes or isothermal bags, stored at chill temperature (4 ± 2 °C) and drawn separately in random sequence for analysis. The analysis started within two hours of the arrival of samples at the laboratory.