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ORIGINAL ARTICLE

A Bayesian modelling framework to estimate *Campylobacter* prevalence and culture methods sensitivity: application to a chicken meat survey in Belgium

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Abstract

Aims: To estimate the true prevalence of *Campylobacter* and the diagnostic sensitivity of routine detection methods by applying a Bayesian modelling approach.

Methods and Results: Results from a Belgium-wide survey of *Campylobacter* contamination in chicken meat preparations ($n = 656$ samples) showed that *Campylobacter* was detected in 24·2% of the samples by enrichment, compared with 41% detected by direct plating. Combining positive results from both methods increased the apparent prevalence to 48·02%. Bayesian model was set up in WinBUGS software, the model estimates *Campylobacter* prevalence as 60% (95% Credibility interval (CI): 47–82%), and the sensitivity of enrichment culture and direct plating as 41% (95% CI: 31–52%) and 69% (95% CI: 50–85%), respectively.

Conclusions: The parallel use of direct plating and enrichment culture adds value for *Campylobacter* detection from chicken meat preparations, but the false-negative results from each culture method must be taken into account.

Significance and Impact of the Study: Monitoring data could be strongly biased by the microbiological techniques used to generate it. To circumvent this bias, we describe an applied Bayesian framework for better interpretation of *Campylobacter* survey data in view of the imperfect test characteristics of routine culture methods.

Introduction

In 2006, *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the EU, as was the case in 2004 and 2005 (EFSA 2007). Scientific evidence has identified chicken meat as one of the most important sources of human campylobacteriosis (Bryan and Doyle 1995; Corry and Atabay 2001; Humphrey *et al.* 2007). The probability of human illness could be reduced by reducing flock prevalence and/or *Campylobacter* concentration on meat. Low levels of *Campylobacter* contamination on meat going to retail calls for initiatives to reduce the contamination level, while high levels of contamination calls for initiatives to reduce the *Campylobacter* prevalence in broilers (WHO 2002; EFSA 2007). Thus, both prevalence and enumeration data on *Campylobacter* contamination levels play an important role in choosing the most efficient control measures.

Two diagnostic issues should be taken into account when incorporating microbiological monitoring data into risk-management decisions, namely (i) the choice of method used in generating such data, and (ii) the fact that all detection methods are of imperfect diagnostic

sensitivity (Gardner 2004). For *Campylobacter* testing, there might be a considerable variation between direct plating and enrichment culture even when testing the same sample. Such variation has been described for testing different foodstuffs and environmental samples; for instance, in 2006, the monitoring data in the Netherlands indicated *Campylobacter* prevalence of 14·5% (199/1368) in broiler meat using enrichment culture. However, the prevalence increased to 34% after combining the recovery of *Campylobacter* by direct plating to the result of recovery by enrichment alone (De Boer and Wit 2007). Musgrove *et al.* (2001) indicated a decrease of 36·7% in detection of *Campylobacter* spp. in chicken caecal samples with enrichment compared with direct culture. In addition, in several cases, Newell *et al.* (2001) showed that strain types were recovered from poultry carcasses by direct plating but not by enrichment, suggesting that enrichment might preferentially select certain *Campylobacter* strains. The second issue concerns the imperfect sensitivity (Se) and specificity (Sp) of the routine culture methods. Scientific evidence shows that detection based on such methods is influenced by *Campylobacter* concentration in the food matrix and the strain's ability to adapt to culture environment (Humphrey and Jørgensen 2006; Rosenquist *et al.* 2007).

In view of the previous diagnostic limitations of routine culture methods, results from a microbiological survey can only provide an idea of the apparent prevalence (AP). In order to estimate the true prevalence (TP), it is necessary to have accurate information on AP, test Se and test Sp (Rogan and Gladen 1978). Values of test Se and Sp have to be obtained either from other data (such as other studies and experimental data) or, in absence of data, from expert opinion. Bayesian statistics allows us to combine such external information (i.e. prior knowledge) with the data at hand (Johnson *et al.* 2000). In other words, a Bayesian framework allows us to assign probability distributions to our prior beliefs and combine these with the data likelihood to yield a posterior probability distribution representing our updated beliefs (Lesaffre *et al.* 2007). Recently, Berkvens *et al.* (2006) described a Bayesian approach using deterministic and probabilistic constraints for the estimation of true disease prevalence and diagnostic test characteristics. This method was validated on data for important zoonotic and veterinary pathogens, such as, porcine cysticercosis (Dorny *et al.* 2004), foot-and-mouth virus in cattle (Goris *et al.* 2007), and cryptosporidiosis in calves (Geurden *et al.* 2008). The aim of this work is to estimate the TP of *Campylobacter* in Belgian chicken meat preparations and the diagnostic sensitivity of routine detection methods by applying a Bayesian modelling approach.

Materials and methods

The survey outline

By definition, chicken meat preparations refer to portioned, cut or minced meat to which spices, seasoning mix, marinate, coating, sauce and other ingredients are added to improve sensory properties or texture, but the cut surface retains the characteristics of fresh meat (Anon. 2004). The sampling frame of chicken meat preparations companies was established based on the Belgian Federal Agency for the Safety of the Food Chain (FASFC) operators list. A targeted-sampling approach was adopted by selecting 11 companies, based on following criteria (EFSA 2006): (i) The companies are distributed across Belgium and in a way that allows the sampling team (two researchers) to visit equally over randomized sampling days each month; (ii) The 11 companies include big, medium and small capacities producers, in order to address diversity in the production chain; (iii) The biggest (three) companies supplying more than 85% of the Belgian distribution chains are included; (iv) The selected companies allow for sampling different batches of portioned and minced products, and different preparation types.

Six hundred and fifty-six samples from the 11 companies were tested between February and November 2007. Samples were distributed over 10 months in order to avoid seasonality bias on prevalence estimate. Descriptive overview of samples is presented in Table 1.

Bacteriology

All samples were tested in parallel by standard direct plating and enrichment culture methods (Anon. 2006a,b). A representative 10-g test portion was homogenized with 90 ml Bolton enrichment broth (CM0983 plus supplement SR183, Oxoid, UK) and testing was carried in parallel as follows: (i) direct plating: 1 ml of the initial homogenate (10^{-1}) was spread plated over four (0·3, 0·3, 0·3, and 0·1 ml) Modified Charcoal Cefoperazon Deoxycholate agar plates (mCCDA) (CM739 plus supplement SR155, Oxoid, UK). From a further serial dilution (10^{-2}), 0·1 ml was spread plated on mCCDA. Plates were incubated microaerobically (5% CO_2, 5% O_2, 5% H_2, and 85% N_2) at 41·5°C and enumerated after 48 h. (ii) enrichment culture: 10 ml from the same sample homogenate were transferred to a sterile tube and incubated microaerobically at 41·5°C, 10 μl were subsequently plated onto mCCDA, and the presence of presumptive *Campylobacter* growth was checked after 24 h and after 48 h.

Enumeration and identification of presumptive *Campylobacter* colonies to genus level were performed

Table 1 Descriptive distribution of the sampled chicken meat preparations (February to November 2007) per companies and product forms

	Minced forms (n = 316)			Portioned forms (n = 340)			
Company ID	Burgers	Minced meat	Sausages	Breast	Legs	Wings	Total
A	5	7	11	9	17	3	52 (7·93%)
B	1	19	5	12	1	0	38 (5·79%)
C	7	1	20	2	13	9	52 (7·93%)
D	12	5	20	8	24	10	79 (12·04%)
E	0	11	14	19	19	7	70 (10·67%)
F	11	13	10	10	1	0	45 (6·86%)
G	2	7	19	32	10	0	70 (10·67%)
H	12	5	17	7	4	0	45 (6·86%)
I	3	18	7	20	16	13	77 (11·74%)
J	9	11	13	17	24	3	77 (11·74%)
K	9	2	10	17	10	3	51 (7·77%)
Total	71 (10·82%)	99 (15·09%)	146 (22·26%)	153 (23·32%)	139 (21·19%)	48 (7·32%)	656 (100%)

according to the ISO 10272:2006 principles (Anon. 2006a,b). In order to assure the true-positive status of a sample, confirmation was performed in two phases: (i) The phenotypic phase: based on the ISO 10272:2006 standard procedure, in brief, colony morphology, oxidase test, strict microaerophilic growth, and microscopic examination after gram staining; (ii) The multiplex PCR phase: up to three isolates (from each positive sample) were identified for *Camp. jejuni* and *Camp. coli* species level, concurrently, according to primers and running protocol described by Vandamme *et al.* (1997).

The data

The principal parameter of interest in the present work is the *Campylobacter* prevalence, and therefore, the results of the direct plating method are coded as present/absent, rather than as count data. Data input for the Bayesian modelling (Fig. 1) is based on the survey testing result output. Detection of *Campylobacter* from chicken meat preparations was 24·2% by enrichment culture, and 41% by direct culture. Combining positive results from both culture methods indicates an apparent *Campylobacter* prevalence of 48·02%. Other details of the survey findings and enumeration results are not relevant for the aim of this paper and will be presented elsewhere (Habib *et al.*, 2008).

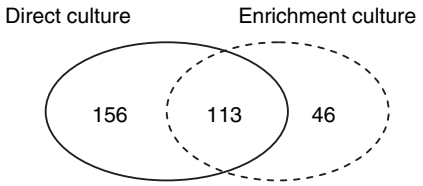

Figure 1 Distribution of *Campylobacter*-positive samples (n = 315) in relation to detection methodology (Total tested samples = 656 chicken meat preparations, from 11 Belgian companies). *Campylobacter* was not detected in 341 samples, those addressed as *Campylobacter*-negative samples.

Bayesian analysis

Two testing procedures were used in parallel, as described earlier, and that implies parallel interpretation of test results (positive = Pos, not-detected = Neg). Letting [Dir] denote direct plating and [Enr] enrichment culture, a sample that yielded a positive result for [Dir] only; e.g. $Dir^{Pos} \cap Enr^{Neg}$, and for [Enr] only; e.g. $Dir^{Neg} \cap Enr^{Pos}$, or for both methods; e.g. $Dir^{Pos} \cap Enr^{Pos}$ is considered a *Campylobacter*-positive sample ($Campy^{Pos}$). Conditional dependence between both culture methods was included in the model based on a multinomial distribution (Berkvens *et al.* 2006) and thus, in theory at least, a total of seven parameters had to be estimated (Table 2).

Estimation of TP and test characteristics was performed in WinBUGS (Spiegelhalter *et al.* 2003). Model selection

Table 2 Bayesian model conditional probabilities between direct plating [Dir] and selective enrichment [Enr] for *Campylobacter* (Campy) detection (Pos/Neg)

Conditional probabilities		
True prevalence	$Pr(Campy^{Pos})$	*th* [1]
Se [Enr]	$Pr(Enr^{Pos} \cap Campy^{Pos})$	*th* [2]
Sp [Enr]	$Pr(Enr^{Neg} \cap Campy^{Neg})$	*th* [3]
Se [Dir]	$Pr(Dir^{Pos} \mid Campy^{Pos} \cap Enr^{Pos})$	*th* [4]*
	$Pr(Dir^{Pos} \mid Campy^{Pos} \cap Enr^{Neg})$	*th* [5]
Sp [Dir]	$Pr(Dir^{Neg} \mid Campy^{Neg} \cap Enr^{Neg})$	*th* [6]
	$Pr(Dir^{Neg} \mid Campy^{Neg} \cap Enr^{Pos})$	*th* [7]

*The statistical notation of Theta (*th*) 4, for example, to be read as: Sensitivity of detection by direct plating (Se [Dir]) = the probability (Pr) of positive samples by direct plating (Dir^{Pos}), giving that (|), samples were contaminated with *Campylobacter* ($Campy^{Pos}$) and (∩) detected by Enrichment (Enr^{Pos}).

and verification was carried out according to criteria described in Spiegelhalter *et al.* (2002) and Berkvens *et al.* (2006). Briefly, the Deviance Information Criterion (DIC) and effective number of parameters estimated (p_D) were used to guide model selection, and the Bayes-*P* value was monitored to assure concordance between prior information and data. Prior information used was mainly based on the absence of false-positives in the survey data, as we assumed that the two-phases confirmation procedure [phenotypic (ISO) + molecular (PCR)] to be perfectly specific. This assumption reduced the original seven parameters (referred to in the model as ϑ [*th*]) to be estimated to four, as the parameters *th*[3], *th*[6], and *th*[7] become equal to unity (Table 2). Furthermore, by applying the Rogan-Gladen equation (Rogan and Gladen 1978) on the chicken meat preparations data at hand, the test Se of the enrichment method could further be limited to values between *c.* 24% ([159/656], Fig. 1, assuming the 341 test negative results are all false-negatives) and *c.* 50% ([159/315], Fig. 1, assuming the 341 test negative results are all true-negatives). The conditional probability referring to the probability of a positive test result for direct plating, given an infected sample and a positive enrichment (*th*[4], Table 2) is represented by a beta distribution with $\alpha = 30$ and $\beta = 12$ (expected value = 113/159 and 95% CI = 0·57–0·85, taking into account the one-stage clustered sampling design).

Finally, based on the chicken meat preparations survey data, there are at least 315/656 true positives, i.e. the TP is at least 45%, and experts indicate 80% as a maximum possible prevalence (Co-authors: L. De Zutter and M. Uyttendaele, personal communication). Both of the consulted experts are Food Microbiology professors at Ghent University, members of the Belgian food safety scientific committee, and have a well-established record of research and field experience regarding *Campylobacter* and its particular situation in Belgium. They justified their opinion for the maximum possible prevalence by weighting between the available national monitoring data and a worst-case precautionary estimate.

The model was run for 50 000 iterations after a burn-in of 5000 iterations. Convergence of each model was assessed by means of the Gelman-Rubin convergence diagnostic graphs in WinBUGS. The final WinBUGS Bayesian model used in our study, and estimation of beta distribution for direct plating sensitivity, are available through the online-supplementary Appendix S1 of this article.

Results

The results of the Bayesian analysis estimates for *Campylobacter* prevalence in chicken meat preparations and the sensitivity of culture methods are given in Table 3.

Table 3 Bayesian estimation of *Campylobacter* prevalence in Belgian chicken meat preparations, and the diagnostic sensitivity of culture methods used

Modelled parameters	Posterior mean	95% Credibility interval
Campylobacter prevalence	0·60	0·47–0·82
Enrichment sensitivity	0·41	0·31–0·52
Direct plating sensitivity	0·69	0·50–0·85

Figure 2 reveals the considerable impact of each culture method on the AP conclusion; prevalence estimation based on enrichment culture alone was the least, compared with direct plating and with the combination of both culture methods. The Bayesian modelling estimation of the TP of *Campylobacter* is higher still because of the fact that the test sensitivities are below 100%.

The priors of the Bayesian model (absence of false-positives and expert opinion on maximum possible prevalence) were validated based on three statistical indices: (i) the DIC; (ii) effective number of parameters estimated (p_D); and (iii) the Bayes-*P* value. More specifically, the values of the DIC and p_D parameters evaluated in the posterior mean of the multinomial probability and found to be in agreement with those evaluated in the posterior mean of the parameters of the model. In applied term, the former parameters provide a statistical verification of the fit between the priors and the survey data. Moreover, an estimated Bayes-*P* value of 0·42 tended towards zero when severe constraints were applied, indicating a good model fit [i.e. all estimates were constrained by a uniform prior on their corresponding models parameters (*th*) to interval ±0·0001% of the posterior estimate].

Discussion

Bayesian statistics becomes exceedingly useful for better interpretation of diagnostic methods performance in both medical and veterinary fields (Enøe *et al.* 2000; Branscum *et al.* 2005). In this study, we present a Bayesian framework to evaluate standard direct plating and enrichment procedures for *Campylobacter* and show the potential of such framework to update a survey-based estimation of pathogen prevalence in chicken meat preparations.

The testing approach recommended by the European Food Safety Authority (EFSA) for a proposed *Campylobacter* monitoring programme in chicken meat preparations (EFSA 2006) was adopted in our survey. This approach is based on performing detection after enrichment and enumeration by direct plating, in parallel, from the same test portion in order to limit subsampling bias. In addition, and in order to assure excluding false-positive test results, confirmation of presumptive-positive

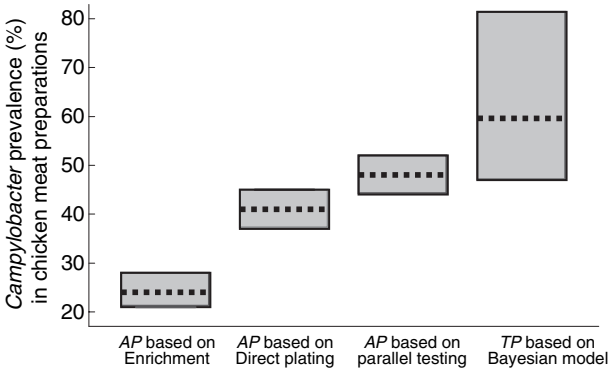

Figure 2 The AP of *Campylobacter* based on enrichment and/or direct culture (represented by the first three boxes on *x*-axis, with 95% binomial CIs), compared with the TP estimation of the Bayesian model (represented by the fourth box on *x*-axis, with 95% credibility interval). The dashed central line inside the boxes represents the mean.

samples was based on a combination of the ISO standard phenotypic procedures and a highly specific PCR test. This two-phase confirmation approach enables the identification of the true-positive status of a sample at both phenotypic and molecular levels. The multiplex PCR we used was evaluated by On and Jordan (2003) to be 93% sensitive and 100% specific for *Camp. jejuni* ssp. *jejuni* identification, and 100% sensitive and 100% specific for *Camp. coli* identification. Recently, Debruyne *et al.* (2008) confirmed the high specificity of this multiplex PCR after testing a collection ($n = 263$) of *Camp. jejuni* and *Camp. coli* field strains. The two phases of results confirmation procedures used in the present survey allows convincingly for excluding false-positive results, and as such, assuming that *Campylobacter*-positive result is a true-positive status is a justifiable assumption. Nevertheless, each culture method introduces a false-negative bias to final results, as 49·5% and 14·6% of *Campylobacter* contaminated samples were tested false-negative by enrichment and by direct culture, respectively (Fig. 1). False-negative by direct plating might indicate cases in which stressed or injured cells were below quantification limit for the direct culture, however, parallel detection after 48-h enrichment enabled their recovery (Gharst *et al.* 2006). On the contrary, campylobacters that has been present and detected by direct plating, but not by parallel enrichment, may have been out-competed during the 48-h enrichment by more vigorous, non*Campylobacter*, members of the microflora in the chicken meat preparations (Musgrove *et al.* 2001).

Previous studies, in concurrent with our chicken meat preparations survey, show that impact of *Campylobacter* detection methods is inevitable, and this need to be taken into account when data are considered nationally and internationally. Belak *et al.* (2006) studied the effect of enrichment (in Bolton broth) on recovery of co-cultured *Camp. jejuni* and *Camp. coli* strains cocktail. Their study showed that *Camp. coli* strains were much more likely to be isolated, while *Camp. jejuni* strains were isolated in significant numbers only when initially inoculated in a very high ratio. Rosenquist *et al.* (2007) concluded that the sensitivity of the qualitative detection (Bolton enrichment and subsequent plating on mCCDA) was significantly lower for samples containing a low concentration of *Campylobacter*. As well, the original study that evaluated CCDA medium indicated that recovery of some *Camp. jejuni* strains (not stressed) was significantly lower on CCDA than on a nonselective agar (Bolton *et al.* 1984).

Our study shows that the parallel use of direct and enrichment culture can be of added diagnostic value for *Campylobacter* detection in chicken meat preparations. The so-called parallel-testing could aid in reducing the imperfect-sensitivity problem associated with routine culture methods. Indeed, parallel-testing increases the proportion of contaminated samples that test positive (the sensitivity), and that was evident in the present survey after combining *Campylobacter* detection by both culture methods (Fig. 2). Nevertheless, applying more than one test to the same sample introduces extra costs and labour variables. As well, parallel-testing approach still did not provide an answer on how many false-negative samples were missed by such an approach (Lesaffre *et al.* 2007). We propose the Bayesian approach to aid better interpretation of *Campylobacter* testing, especially when testing data are the base on which a public health or risk analysis decision to be taken. The Bayesian framework we applied for the case of *Campylobacter* in Belgian chicken meat preparations reflects very closely the real nature of the scientific method, and it was possible to validate the subjectivity in the prior opinion through the use of a set of statistics and indices (DIC, p_D, and Bayes-*P*) (Berkvens *et al.* 2006). The need for informative prior information should not be seen as a disadvantage; it is quite common in bioscience applications to have some form of expert opinion, and Bayesian methods allow this to be incorporated (Gustafson 2005). Moreover, external information and expert opinion are important component in many of the already available microbiological risk assessment models for *Campylobacter* in poultry meat chain in different European settings (Rosenquist *et al.* 2003; Uyttendaele *et al.* 2006; Nauta *et al.* 2007).

The present study adds to the available, limited, knowledge about test characteristics of *Campylobacter* detection methods. Recently, Woldemariam *et al.* (2008) estimated sensitivity of direct plating from broiler faecal culture to be 21% (95% CI: 12–31%) and 23% (95% CI: 13–60%) using two different Bayesian models. Their estimation of direct plating sensitivity for broiler caecal samples was

64% (95% CI: 37–89%) and 66% (95% CI: 39–90%) using two modelling approaches. On the contrary, the overall sensitivity of the *Campylobacter* enrichment culture was estimated by Rosenquist *et al.* (2007), through a collaborative study on a Nordic standard protocol, as 82·8% (95% CI: 78·4–86·6%). Our Bayesian estimation for the sensitivity of enrichment culture is much lower (nearly the half) than what concluded by the former collaborative study. However, it should be taken into consideration that the Nordic standard collaborative study is based on testing 'artificially inoculated' samples, and sensitivity estimate to be drawn from such study design is not necessary a reflection of the real diagnostic sensitivity while testing different 'naturally contaminated' food matrixes. Mentioning that, it is important to note that our Bayesian estimation of the sensitivity of enrichment and direct plating methods (Table 3) should not be used as general values, but that can be used, ideally, for *Campylobacter* detection in chicken meat preparations and other resembling chicken meat matrixes, whenever appropriate.

In conclusion, it is important to recognize that the data output from a monitoring programme could be strongly biased by the microbiological techniques used to generate it. Our experience with *Campylobacter* in chicken meat preparations shows that a combination of direct plating and enrichment culture, in parallel, would benefit the prevalence estimation of *Campylobacter* in such chicken meat matrix. We applied a modelling framework involving the use of appropriate statistical indices to assure that the prior opinions are in concordance with data. We recommend the consideration of Bayesian framework to help better interpretation of *Campylobacter* survey data in view of the imperfect test characteristics of the routine culture methods.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1 (A) Bayesian model used in WinBUGS to estimate true prevalence and test characteristics. (B) Estimation of Beta distribution to represent the sensitivity of direct plating (*th*[4] in the model).

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