Epidemiol. Infect. (2015), **143**, 298–307. © Cambridge University Press 2014
doi:10.1017/S0950268814000454

Bayesian analysis of culture and PCR methods for detection of *Campylobacter* spp. in broiler caecal samples

M. E. ARNOLD[1]*, E. M. JONES[2], J. R. LAWES[3], A. B. VIDAL[4], F. A. CLIFTON-HADLEY[4], J. D. RODGERS[4] AND L. F. POWELL[3]

[1] *Animal Health and Veterinary Laboratories Agency, Sutton Bonington, Loughborough, UK*
[2] *Biomathematics and Statistics Group, Animal Health and Veterinary Laboratories Agency, Surrey, UK*
[3] *Epidemiology and Risk Group, Animal Health and Veterinary Laboratories Agency, Surrey, UK*
[4] *Department of Bacteriology, Animal Health and Veterinary Laboratories Agency, Surrey, UK*

Received 9 July 2013; Final revision 7 February 2014; Accepted 10 February 2014; first published online 20 March 2014

SUMMARY

The objective of this study was to estimate the sensitivity and specificity of a culture method and a polymerase chain reaction (PCR) method for detection of two *Campylobacter* species: *C. jejuni* and *C. coli*. Data were collected during a 3-year survey of UK broiler flocks, and consisted of parallel sampling of caeca from 436 batches of birds by both PCR and culture. Batches were stratified by season (summer/non-summer) and whether they were the first depopulation of the flock, resulting in four sub-populations. A Bayesian approach in the absence of a gold standard was adopted, and the sensitivity and specificity of the PCR and culture for each *Campylobacter* subtype was estimated, along with the true *C. jejuni* and *C. coli* prevalence in each sub-population. Results indicated that the sensitivity of the culture method was higher than that of PCR in detecting both species when the samples were derived from populations infected with at most one species of *Campylobacter*. However, from a mixed population, the sensitivity of culture for detecting both *C. jejuni* or *C. coli* is reduced while PCR is potentially able to detect both species, although the total probability of correctly identifying at least one species by PCR is similar to that of the culture method.

Key words: *Campylobacter*, Bayesian method, diagnostic test evaluation, foodborne zoonoses.

INTRODUCTION

Campylobacter spp. is the most common bacterial cause of human gastrointestinal disease in most developed countries [1]. *C. jejuni* is the most common species in human campylobacteriosis followed by *C. coli* [2, 3]. Both species are frequently found in the alimentary tracts of a wide range of animals [4] with *C. jejuni* being most associated with the contamination of poultry flocks and poultry products, while *C. coli* is found predominantly in pigs [5, 6].

Poultry and poultry products remain one of the most important sources of human campylobacteriosis. A baseline survey carried out at European Union (EU) level, found the prevalence of *Campylobacter*-colonized broiler batches was 71·2% overall but this varied greatly between Member States (MS) from 2% to 100% [7]. *C. jejuni* and *C. coli* were found in 60·8% and 41·5%, respectively, of positive batches but the species distribution was highly variable across the EU. *C. jejuni* was the most common in 19 MS

* Author for correspondence: M. E. Arnold, Animal Health and Veterinary Laboratories Agency, The Elms, College Road, Sutton Bonington, Loughborough LE12 5RB, UK.
(Email: mark.arnold@ahvla.gsi.gov.uk)

while in seven MS the frequency of *C. coli* ranged from 76·1% and 97·7% of the species identified. The reason for these large differences in the species prevalence in broiler flocks is unknown. However, microbiological methods based on culture and biochemical identification do not provide a true measure of the prevalence of the different species present in a sample as both the selective media used and the incubation atmosphere may have an impact on the species recovered [8–11], and at best provide an approximation of the prevalence of these species in broiler flocks.

Polymerase chain reaction (PCR)-based methods have been applied for the detection of *Campylobacter* spp. directly from caecal contents, and some methods are able to identify both *C. jejuni* and *C. coli* in samples with greater sensitivity than conventional culture methods [12]. The accurate estimation of the true prevalence of these two species in broiler flocks may allow a better understanding of their epidemiology in such populations and assessment of their relative importance for human infection.

During 2007–2009 a UK-wide, 3-year survey of broiler flocks was conducted to estimate the prevalence of *Campylobacter*-infected batches of birds at slaughter [13] by direct culture of caecal samples. A prerequisite for the determination of the actual prevalence of each *Campylobacter* species and hence their epidemiology is knowledge of the sensitivity and specificity of the culture test for these organisms. These performance criteria should be evaluated by comparison to a perfect (gold standard) reference test; however, for *Campylobacter* such a gold standard is not available to accurately determine infection status. As the true status of each batch was unknown, this created difficulties in assessing the true sensitivity and specificity of the caecal culture test. In recent years, the use of Bayesian modelling to estimate the sensitivity and specificity of a diagnostic test has been applied for this purpose [14–16]. For a Bayesian model to infer the sensitivity and specificity of a culture method a second conditionally independent test needs to be applied in parallel to ensure there are, at least, as many degrees of freedom in the data as there are parameters requiring estimation [14]. To this end, a PCR test was applied to the caecal samples in parallel with culture. The objective of this study was to apply a Bayesian framework to evaluate standard direct culture and PCR for the detection of *C. jejuni* and *C. coli* in broiler caecal samples, and use this framework to estimate the true prevalence of these species in broiler flocks in the UK.

MATERIALS AND METHODS

The *Campylobacter* status of a sample was considered 'mixed' when both *C. jejuni* and *C. coli* were detected in a sample. Samples, where *C. jejuni* alone was present, were denoted as '*C. jejuni*' (i.e. a non-mixed sample), and similarly for '*C. coli*'.

Sample and data collection

Caecal samples were collected from broiler slaughter batches as part of a 3-year randomized national prevalence survey [13]. Briefly, per slaughter batch, a single caecum was collected for sampling from 10 different broilers at the time of evisceration. Samples were selected according to the month of sampling and flock depopulation status (i.e. whether birds had previously been taken from the flock, sometimes known as 'thinning'). Caecal content was obtained from the caecum, stored at 4 °C and tested within 1 week after collection by bacteriological culture and PCR.

Culture of pooled caecal sample

The method used for the detection and speciation of *Campylobacter* spp. in caeca was in accordance with the technical specifications set out in Annex I of Commission Decision 2007/516/EC and as reported previously [13]. For each slaughter batch, caecal contents were pooled from each of the ten caeca individually by homogenizing 0·02 g from each caecum in 2 ml phosphate buffered saline (PBS) (0·1 m, pH 7·2). A 10 μl volume of this was plated on mCCDA agar (CM739 base with SR155 supplement; Oxoid, UK) and incubated at 41·5 ± 1 °C microaerobically (84% N_2, 10% CO_2, 6% O_2). Plates were examined at 24 h and 48 h for grey flat, irregular and spreading colonies typical of *Campylobacter* spp. [17]. Up to five suspect colonies were subcultured micro-aerobically as described onto 7% sheep blood agar with 0·1% cyclohexamide (CM0055, Oxoid) before confirmation and species identification based on phenotypic methods described in ISO 10 272–1:2006(E) [18]. The detection limit for the culture method for caecal samples using mCCDA has been estimated as 10^2 c.f.u./g [19] and for the PCR as 10^5 c.f.u./g [12].

Each sample, was considered culture positive if at least one colony recovered was confirmed as thermophilic *Campylobacter* spp. Speciation tests were performed on one single colony per positive batch as described previously [13].

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

DNA extraction and PCR test

Extractions were performed within 7 days of caecal collection using an ExtractMaster™ Fecal DNA Extraction kit (Epicentre Biotechnologies, USA). A 250 μl sample of caecal suspension in 750 μl PBS was centrifuged for 5 min at 13 000 ***g*** and the pellet was re-suspended in 5 μl Tris buffer (1 m, pH 8) and extraction continued following the manufacturer's protocol. DNA preparations were stored at −20 °C until PCR testing for *C. jejuni* and *C. coli* as described previously [12]. A cycle threshold (C_t) of between 10 and 32 was viewed as a positive result for either the *mapA* probe (*C. jejuni*) or *ceuE* probe (*C. coli*). A negative result was recorded for *mapA* and *ceuE* probes if the C_t value was >32 and the C_t value of the internal amplification control (IAC) probe was <40; however, when there was no C_t value for the IAC probe the result was invalid.

Statistical methods

All statistical analysis was conducted in WinBUGS 1.4, using a modified version of the approach used in [14], in which a Bayesian method is proposed for estimating the sensitivity and specificity of two tests applied to two populations in the absence of a gold standard. In this study, there were four populations:

(1) First batch removed from flock, non-summer (October–March).
(2) First batch removed from flock, summer (June–September).
(3) Previously partly depopulated, non-summer (October–March).
(4) Previously partly depopulated, summer (June–September).

Each pooled caecal sample was tested by PCR and culture. As the culture result was based on a single colony, it was only possible to detect one species of *Campylobacter*, although both *C. jejuni* and *C. coli* may have been present in the sample. In light of this, there were 12 possible outcomes for each sample tested.

For each population $k = 1, \ldots, 4$, the observations were condensed into a descriptive 12-dimensional vector $\mathbf{y_k}$. These vectors, for each of the four populations, were assumed to have independent multinomial sampling distributions,

$$\mathbf{y_k} \sim \text{multinomial}(n_k, \mathbf{p_k}),$$

where n_k represents the number of observations in population k, and $\mathbf{p_k}$ denotes the vector of probabilities associated with each of the 12 possible outcomes.

Elements of the vector $\mathbf{p_k}$ are defined by weighting the sensitivity and specificity with the proportion of samples deriving from each of the four possible population statuses: *C. jejuni* only (π_j), *C. coli* only (π_c), both species (π_m), or *Campylobacter*-free, with the sum of the prevalence estimates for each species constrained to be no more than 1. A detailed description of the elements of the vector $\mathbf{p_k}$ is given in the Supplementary material (Table S1).

It was assumed that the sensitivity of PCR to detect *C. jejuni* and *C. coli* would be unaffected by whether the sample contained both species. Similarly, the sensitivity of culture was estimated for both *C. jejuni* and *C. coli* for samples with separate estimates when only one species of *Campylobacter* was present and for mixed samples. Furthermore, for culture the sensitivity of *Campylobacter* spp. is likely to be affected by the relative proportions of mixed species samples in the population, therefore account was taken of the relative proportions of mixed/*C. jejuni*/*C. coli* samples in each population.

Priors

The model was initially run with non-informative priors throughout, except for specificity of culture. The sensitivity of the model results to the choice of priors was examined by (i) running the model with informative priors for the sensitivity of culture (as given in Table 1) and (ii) with non-informative priors for all parameters. Where informative priors were used, beta-distributed priors were based on previous studies [9, 12, 20] (Table 1). BetaBuster software (University of California, Davis, USA) was used to determine the parameters for each variable.

Data for the prior elicitation for the sensitivity of culture to detect *C. jejuni* and *C. coli* were derived from a previous study, where caeca, boot swabs and faecal samples were collected in parallel from 36 flocks [9]. In this study, Bayesian methods were used to estimate the sensitivity of each of the sampling methods to detect *C. jejuni* and *C. coli*, where the samples were from a mixture of flocks with both species or *C. jejuni* alone (no flocks had *C. coli* only). The required prior for the sensitivity of culture to detect *C. coli* in a mixed sample, λ_{cm}, was available from [9]. The priors for the sensitivity of culture to detect *C. jejuni* in a non-mixed sample (λ_{jj}), and a mixed

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

Table 1. *Priors used for the Bayesian model to estimate the sensitivity and specificity of PCR and caecal culture for detection of* Campylobacter *in broiler chickens*

Parameter	Description	Prior	Source
θ_{jj}	Sensitivity of PCR to detect *C. jejuni*	Beta(1,1)	Non-informative
θ_{cc}	Sensitivity of PCR to detect *C. coli*	Beta(1,1)	Non-informative
$1-\theta_{jf}$	Specificity of PCR (for *C. jejuni*)	Beta(1, 1)	Non-informative
$1-\theta_{cf}$	Specificity of PCR (for *C. coli*)	Beta(1, 1)	Non-informative
λ_{jj}	Sensitivity of culture to detect *C. jejuni* (with no *C. coli* present)	(i) Beta(1,1) (ii) Beta(17·7, 1·86)	(i) Non-informative (ii) From Vidal *et al.* [9] Mode of 95·1%, 95% sure >77·9
λ_{cc}	Sensitivity of culture to detect *C. coli* (with no *C. jejuni* present)	(i) Beta(1,1) (ii) Beta(17·7, 1·86)	(i) Non-informative (ii) Same prior as λ_{jj}
λ_{jm}	Sensitivity of culture to detect *C. jejuni* in mixed sample	(i) Beta(1,1) (ii) Beta(2·46, 4·04)	(i) Non-informative Derived from Vidal *et al.* [9] 95% sure >11·1%, mode of 32·5%
λ_{cm}	Sensitivity of culture to detect *C. coli* in mixed sample	(i) Beta(1,1) (ii) Beta(7·36, 5·99)	(i) Non-informative (ii) Derived from Vidal *et al.* [9] 95% sure >33·0%, mode of 56%
$1-\lambda_{jf}$	Specificity of culture for *C. jejuni*	Beta(102·4, 3·1)	Woldemarium *et al.* [20] 95% sure >94% and mode of 98%.
$1-\lambda_{cf}$	Specificity of culture for *C. coli*	Beta(102·4, 3·1)	Woldemarium *et al.* [20] 95% sure >94% and mode of 98%.

Table 2. *Number of* Campylobacter*-positive batches from PCR by species (C_t<32 to be designated positive) and the respective result of culture methods, applied to the four broiler populations, based on caecal samples (taken from broilers at slaughter as part of a national prevalence survey)*

Population: description (number of batches)	PCR result	Culture result		
		C. jejuni	*C. coli*	Negative
1. First batch removed (November–March) (n=91)	*C. jejuni*	22	0	1
	C. coli	0	3	1
	Mixed	3	3	1
	Negative	17	2	38
2. First batch removed (June–September) (n=59)	*C. jejuni*	23	1	2
	C. coli	2	2	1
	Mixed	6	10	3
	Negative	1	0	8
3. Previously partly depopulated (November–March) (n=179)	*C. jejuni*	87	4	14
	C. coli	1	3	0
	Mixed	11	18	1
	Negative	20	1	19
4. Previously partly depopulated (June–September) (n=107)	*C. jejuni*	47	2	2
	C. coli	0	6	0
	Mixed	11	21	2
	Negative	9	3	4

sample (λ_{jm}), were obtained by splitting the positive flocks into *C. jejuni* only and flocks with both *C. jejuni* and *C. coli*, and estimating the sensitivity of culture of *C. jejuni* in each case. For the sensitivity of culture of mixed samples, a Dirichlet distribution was used to represent the priors, to ensure that λ_{jm}, λ_{cm} were each between 0 and 1 and that the sum of the probabilities λ_{cm}, λ_{jm}, and the probability of a false-negative mixed sample ($1-\lambda_{cm}-\lambda_{jm}$) summed to 1 (see Supplementary material for further details and choice of priors).

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

Table 3. *Number of* Campylobacter-*positive batches from PCR (for $C_t<32$ and $C_t<36$) and culture methods applied to the four broiler populations, based on caecal samples (taken from broilers at slaughter as part of a national prevalence survey)*

Population: description (no. of batches)	Season	Species	PCR positives		Culture positive
			$C_t<32$ for positive	$C_t<36$ for positive	
1. First batch removed ($n=91$)	Non-summer (November–March)	*C. jejuni*	23 (25·3%)	26 (28·6%)	43 (47·3%)
		C. coli	4 (4·4%)	5 (5·5%)	8 (8·8%)
		Mixed	7 (7·7%)	9 (9·9%)	n.a.
		Total	34 (37·4%)	40 (44·0%)	51 (56·0%)
2. First batch removed ($n=59$)	Summer (June–September)	*C. jejuni*	26 (44·1%)	24 (40·7%)	32 (54·2%)
		C. coli	5 (8·5%)	4 (6·8%)	13 (22%)
		Mixed	19 (32·2%)	22 (37·3%)	n.a.
		Total	50 (84·7%)	50 (84·7%)	45 (76·2%)
3. Previously partly depopulated ($n=179$)	Non-summer (November–March	*C. jejuni*	105 (58·7%)	98 (54·7%)	119 (66·5%)
		C. coli	4 (2·2%)	5 (2·8%)	26 (14·5%)
		Mixed	30 (16·8%)	44 (24·6%)	n.a.
		Total	139 (77·7%)	147(81·6%)	145 (81·0%)
4. Previously partly depopulated ($n=107$)	Summer (June–September)	*C. jejuni*	51 (47·7%)	49 (45·8%)	67 (62·6%)
		C. coli	6 (5·6%)	4 (3·7%)	32 (29·9%)
		Mixed	34 (31·8%)	45 (42·1%)	n.a.
		Total	91 (85·0%)	98 (88·8%)	99 (92·5%)

n.a., Not applicable.

We assumed that PCR would correctly classify the *Campylobacter* species. Due to the possibly imperfect nature of the hippurate test for speciation, some misclassification was allowed for culture in the model. A similar approach was adopted as for setting priors for sensitivity of culture of mixed samples, i.e a Dirichlet distribution was used for λ_{jj}, λ_{jc}. It was assumed that $\lambda_{cj}=\lambda_{jc}$, i.e. an equal probability of misclassification for either species. We also assumed vague (uniform in the range 0–1) priors for the specificity and sensitivity of PCR, and for the batch prevalence for each group of birds (season and thinning status).

The cut-off value used for designating a sample positive by PCR was $C_t \leqslant 32$ [12]. However, the Bayesian model was also used to explore the impact of increasing the cut-off to 36 on the sensitivity and specificity of PCR to detect each *Campylobacter* species.

RESULTS

Overall, samples from 436 slaughter batches, originating from 22 abattoirs in the UK were tested by PCR and culture methods. There were very few samples with only *C. coli* present; the majority also contained *C. jejuni* detected by PCR (the number of mixed samples greatly outnumbered the number of *C. coli* PCR positives). Furthermore, PCR appeared to be less sensitive than culture, with a much higher number of PCR-negative samples being positive for culture (19, 1, 21 and 12 for populations 1–4, respectively; Table 2) compared to samples that were negative by culture but positive by PCR (3, 6, 15 and 4 for populations 1–4, respectively; Table 2). The apparent prevalence of *C. jejuni* was markedly higher (range 47·3–66·5%) than that of *C. coli* (range 8·8–22%) in all four populations tested by culture (Table 3).

There was also evidence of a higher sensitivity of culture to detect *C. coli*, in line with the prior for relative sensitivity of culture to detect both species in a sample, as for mixed samples a total of 31 (3 + 6+11 + 11) were positive for *C. jejuni* by culture, compared to 52 (3 + 10 + 18 + 21) positive for *C. coli* by culture (Table 2).

Estimates from Bayesian model

An important impact of season on the true prevalence estimates of *C. coli* was observed. The estimated proportion of batches containing *C. coli* (the sum of the *C. coli*-only and mixed batches, Table 4) increased

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

Table 4. *Estimated prevalence of* Campylobacter *in batches of broilers from four populations, using a Bayesian model applied to caecal sampling data**

Population: description	Season	Species	Percentage infected (median)	95% CrI
1. First batch removed	Non-summer (November–March)	*C. jejuni*	42·7	(31·2–53·7)
		C. coli	4·1	(0·5–10·6)
		Mixed	9·6	(4·3–17·5)
		Total	56·4	
2. First batch removed	Summer (June–September)	*C. jejuni*	40·4	(27·3–53·8)
		C. coli	2·7	(0·1–10·6)
		Mixed	41	(28·4–55·3)
		Total	84·1	
3. Previously partly depopulated	Non-summer (November–March)	*C. jejuni*	66·7	(58·8–73·9)
		C. coli	0·8	(0–3·4)
		Mixed	21·4	(15·2–28·6)
		Total	88·9	
4. Previously partly depopulated	Summer (June–September)	*C. jejuni*	54·7	(44·2–65·2)
		C. coli	3·7	(0·2–11)
		Mixed	40	(29·6–51·2)
		Total	98·4	

CrI, Credible interval.
* A threshold value of $C_t < 32$ was used for a positive designation by PCR.

from 13·7% in non-summer to 43·7% during the summer months for flocks that had not been previously partly depopulated and from 22·2% in non-summer to 43·7% during the summer months for previously depopulated flocks. For *C. jejuni*, the impact of season appeared to be important only for batches that had not been previously partly depopulated; in this case the prevalence decreased from 81·4% in the summer to 52·3% in non-summer. For batches that had been previously partly depopulated there was very little change in *C. jejuni* prevalence between non-summer (88·1%) and summer (94·7%). The increase in *C. coli* during the summer months was mainly due to an increase in mixed positive samples with the corresponding reduction in the proportion of samples that contained only *C. jejuni* in batches of chickens from the previously depopulated flocks.

There was also an impact of the depopulation status of the flock on the true prevalence estimates of *Campylobacter* (Table 4). In the non-summer months, there was a marked difference in the total of *Campylobacter* prevalence between batches that were the first to be removed from the flock (56·4%) and previously partly depopulated batches (88·9%). In the summer, the *Campylobacter* prevalence of first removed batches was very high (84·1%), and even though the prevalence increased to 98·4% for previously partly depopulated batches, the relative change was much smaller than for non-summer.

Given a batch with only one species of *Campylobacter* present, culture had high sensitivity (97·5% and 83·3% for *C. jejuni* and *C. coli*, respectively), with a lower sensitivity for PCR (81·4% and 86·51% for *C. jejuni* and *C. coli*, respectively) (Table 5). The model results suggested a lower specificity of PCR (3·4% and 2·9% probability of a negative sample being designated as *C. jejuni* and *C. coli*, respectively) compared to culture (2·2.% and 1·0% for *C. jejuni* and *C. coli*, respectively). When analysis used a threshold of $C_t<36$ to designate a PCR test positive, there was a small increase in sensitivity and a similar decrease in specificity (see Supplementary Table S2).

For mixed samples, the results indicated a bias in favour of culture detecting *C. coli* in preference to *C. jejuni*, with a 59·9% likelihood of a mixed sample being positive for *C. coli* compared to 37·3% of being positive for *C. jejuni* (Table 5).

There were differences of the order of <1% between the sensitivity of culture to detect *Campylobacter* spp. for each of the four sub-populations 1–4 (Table 6). The sensitivity of culture to detect *C. jejuni* was equal to or higher than that to detect *C. coli*; when there was a difference, it was more marked in the

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

Table 5. *Bayesian model estimates (plus 95% credible intervals) of the true sensitivity and specificity of PCR (using a threshold of $C_t < 32$ for a positive designation) and culture for detection of* Campylobacter *by species (applied to caecal samples from broilers)*

	Probability of PCR result, % (95% CrI)		Probability of culture result, % (95% CrI)	
Campylobacter in sample	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*
C. jejuni only	81·4 (76·7–85·7)		97·5 (93·3–99·6)	1·4 (0·06–5·2)
C. coli only		86·1 (75·6–97·0)	1·4 (0·06–5·2)	83·3 (35·8–98·3)
Mixed	Assumed same as for non-mixed samples		37·3 (28·1–47·2)	59·9 (49·9–69·3)
None	3·4 (0·2–11·2)	2·9 (0·2–9·9)	2·2 (0·12–8·2)	1·2 (0·06–4·3)

CrI, Credible interval.

Table 6. *Sensitivity of culture to detect* Campylobacter *spp.,* C. jejuni*, and* C. coli *in each of the four sub-populations sampled in the study (i.e. taking into account the proportion of mixed samples cultured)*

	Bayesian estimate of culture sensitivity (95% CrI)		
Population	*Campylobacter* spp.	*C. jejuni*	*C. coli*
1. First batch removed (November–March)	0·96 (0·91–0·99)	0·86 (0·77–0·93)	0·66 (0·52–0·79)
2. First batch removed (June–September)	0·97 (0·93–0·99)	0·67 (0·57–0·76)	0·61 (0·51–0·71)
3. Previously partly depopulated (November–March)	0·97 (0·94–0·99)	0·83 (0·77–0·87)	0·61 (0·51–0·7)
4. Previously partly depopulated (June–September)	0·97 (0·93–0·99)	0·72 (0·64–0·79)	0·62 (0·52–0·71)

CrI, Credible interval.

non-summer months, where the sensitivity of culture was 20% higher for *C. jejuni* than for *C. coli*, whereas for the summer months the difference was 5–10%, both for 'first batch removed' and for 'previously depopulated' batches.

Sensitivity of posterior estimates to prior assumptions

There was little difference in the results between the model with informative priors for culture sensitivity and specificity and results with only informative priors for culture specificity (the baseline model), except for the sensitivity of culture for *C. coli* (i.e. non-mixed) samples. The estimate of sensitivity for *C. coli* increased from 0·83 to 0·92 (but both with wide credible intervals) when informative priors were used for its sensitivity (Supplementary Table S3).

When non-informative priors were used for all parameters, there were few differences between the parameters compared to the baseline model, except for: (i) the estimate of the sensitivity of PCR to detect *C. jejuni* (in non-mixed samples) increased from 0·81 to 0·87, and (ii) the estimate of the specificity of culture reduced from 0·98 to 0·74.

DISCUSSION

This study has estimated the sensitivity and specificity of direct culture on mCCDA and a real-time PCR for detection of *C. jejuni* and *C. coli* from broiler caecal samples. Direct culture on mCCDA was the diagnostic test used to determine *Campylobacter* prevalence in a recent 3-year survey [13] and was also used in the EU baseline survey [21]. Results indicate imperfect sensitivity and specificity of both PCR and culture, with potentially important differences in sensitivity of culture by species. This imperfect sensitivity suggests a likely underestimation of the prevalence of *Campylobacter* in the UK survey (see Table 6) where an overall prevalence of 79·2% was observed with 74·8% and 25·1% of the positive broiler batches being contaminated with *C. jejuni* and *C. coli*, respectively [13]. The overall sensitivity of culture for both *C. jejuni* and *C. coli* varies between the sub-populations considered in the present study, probably due to differences in the relative prevalence and contamination levels of *C. jejuni*/*C. coli*/mixed samples in each sub-population. Estimation of the overall sensitivity of culture for each species indicates that there will be a greater underestimation of the *C. coli*

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

prevalence than that of *C. jejuni* (Table 6). For samples where both species are present, there will be greater underestimation of *C. jejuni* than *C. coli* (Table 6).

Specificity of culture was found to be close to that obtained by a previous study (~98%) [20], although it was higher for *C. coli* than *C. jejuni*. One possible cause of false positives and thus imperfect specificity of culture is the misclassification of species due to the hippurate test. The sensitivity of culture estimated in the present study is higher (64%) than that reported in [18] but comparison between the two studies is difficult owing to markedly different methodologies used for collection, transportation and culture of the samples. Other studies [10, 19] have also reported lower sensitivity of culture, but used a different matrix and therefore the culture tests are not directly comparable with the present study.

The analysis of *Campylobacter* results by species prompts the need for a more complicated expression for the likelihood of the data compared to the standard two 2-test model [14]. Due to the larger number of prevalence and test sensitivities that need to be estimated in the Bayesian model here, it was considered important to obtain informative parameters for some of the parameters, in order to assist with model identifiability. The sensitivity analysis indicated that models with informative priors for the sensitivity and specificity of culture produced estimates with reasonably close agreement to those obtained using non-informative priors. One of the main differences in using non-informative priors was that the estimate of the sensitivity of culture to detect *C. coli* in a non-mixed sample was lower (83%) than the estimate with an informative prior (92%). This sensitivity of the estimate of *C. coli* culture to the choice of prior is likely to be due to the relatively small number of *Campylobacter*-positive samples that contain only *C. coli* and not *C. jejuni*; out of 436 samples only 19 were identified as *C. coli*-only positives by PCR (at a cut-off of 32). This leads to difficulties in robust statistical inference for the sensitivity of culture to detect *C. coli* from the present study data alone. The estimate for the specificity of culture for *C. jejuni* when it was the only species present was also influenced by the choice of prior; with non-informative priors it dropped from 0·97 to 0·74. There is a potential lack of identifiability in the infection status of samples that were *C. jejuni* positive for culture but *C. jejuni* negative for PCR. However, the estimate of specificity of 0·74 is not credible in the light of previous work [20] or the recent EU survey on *Campylobacter* in broilers, with apparent prevalence in pooled caecal samples being as low as 2% in one MS [21].

The aim of the inclusion of an informative prior scenario as part of the sensitivity analysis was to determine how sensitive the model results were to changes in the priors. It turned out that the priors generated for the sensitivity of culture for mixed samples from a previous study [9] were very close to the posteriors when non-informative priors were used, indicating good agreement with the present study.

A very high proportion of flocks detected as having *C. coli* by culture were co-infected with *C. jejuni* by PCR testing. This finding may indicate that in most cases where *C. coli* has colonized a flock, it does so at a higher level than *C. jejuni* at time of slaughter, similar to the findings of [22], albeit with a limited number of *C. coli* and *C. jejuni* strains. When both species are present, *C. coli* is generally present in higher numbers and hence more likely to be the species detected by culture (J. Rodgers, AHVLA, unpublished data). This might suggest a different epidemiology for the two species such as different contamination or multiplication rates; once *C. coli* colonization has occurred, *C. jejuni* may also colonize the flock but does not reach such high levels when *C. coli* is present. Further difference in the epidemiology of the two species is reflected by the impact of whether a batch was the first to be removed from the flock, and the impact of seasonality on the prevalence of *Campylobacter*. For *Campylobacter* spp. and *C. jejuni* these were consistent with previous studies, where prevalence was higher in thinned flocks, and in the summer [13]. However, for *C. coli*, there was little difference in prevalence between the first batch and previously partly depopulated flocks, although there was a clear seasonal difference. Thinning or partial depopulation does not seem to increase the prevalence of *C. coli*; this may imply that other factors affect flock colonization with this species such as environmental and climate conditions. It may also indicate different times of colonization as an earlier colonization would allow more time for *C. coli* to become established and predominate in the flock. Further investigations are required to explore this hypothesis.

Although high, the sensitivity of PCR was lower than the culture method. This is possibly due to the intrinsic higher limit of detection of the PCR (>10^5 c.f.u./g) [12] compared to culture (>10^2 c.f.u./g) [19] and therefore caecal samples with lower concentrations of bacteria will not be detected by PCR.

https://doi.org/10.1017/S0950268814000454 Published online by Cambridge University Press

By contrast, PCR exhibited higher specificity over culture than that of 80% reported previously [12], but it should be noted on a much smaller number ($n = 52$) of samples. Furthermore, the latter study only tested a single flock population and was not able to apply Bayesian methods in the absence of a gold standard, therefore the adoption of culture as the reference standard may have influenced their results. The higher specificity found here is consistent with our experience of applying PCR to a large number of samples in *Campylobacter* surveys, where there were fewer culture-negative samples testing positive by PCR (data not shown). Furthermore, test validation of the PCR with several bacterial species underlines its high specificity [23]. A high specificity (96·2%) of a real-time PCR relative to culture as the reference standard was also reported when applied to faeces from experimentally infected pigs [24], and, by extension from above, the actual specificity could be even higher if some samples were false-negative by culture.

It would interesting to explore further the optimal choice of C_t threshold at which to determine a sample as positive by PCR, since changing this did have an effect on the sensitivity and specificity of the test. One method of doing this would be to use recently developed Bayesian methods that are able to analyse the PCR data without using a specific cut-off, i.e. the actual C_t value is used in the analysis [25]. Such an approach would result in a more powerful dataset, since the model will have information on definite and borderline positives for each species, and provide further strength to the model inference.

In conclusion, the season of sampling had an important impact, especially for *C. coli*, which was more prevalent in the summer while for *C. jejuni* the effect of season was only marked for the batches first removed from the flock. Previous partial depopulation of the flock also had an important impact, with lower prevalence of both *Campylobacter* species (with a larger change for *C. jejuni*) for batches that were the first birds removed from the flock than for previously partly depopulated batches. Culture was more sensitive than PCR for both species in samples derived from populations infected with a single species of *Campylobacter* but its sensitivity was reduced for *C. jejuni* or *C. coli* in mixed populations although it was less marked for the latter. The PCR method is potentially able to detect both species in mixed samples but the total probability of correctly identifying at least one species by this method was similar to culture.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814000454.

ACKNOWLEDGEMENTS

The authors thank the Department for Environment, Food and Rural Affairs (Defra) for funding the study (project OZ0613) and the abattoirs and the poultry companies for participating in this study. The authors also thank Meat Hygiene Service colleagues for collecting the samples and colleagues from the Epidemiology and Risk Group and the Department of Bacteriology at the AHVLA for their involvement with the survey.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Olson CK, *et al.*** Epidemiology of *Campylobacter jejuni* infections in industrialized nations. In: Nachamkin I, Szymanski CM, Blaser MJ, eds. *Campylobacter*. Washington: AMS Press, 2008, pp. 163–189.
2. **Gillespie IA, *et al.*** A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. *Emerging Infectious Diseases* 2002; **8**: 937–942.
3. **Tam CC, *et al.*** *Campylobacter coli* – an important foodborne pathogen. *Journal of Infection* 2003; **47**: 28–32.
4. **Newell DG, Fearnley C.** Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology* 2003; **69**: 4343–4351.
5. **Miller WG, *et al.*** Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology* 2006; **152**: 245–255.
6. **Thakur S, *et al.*** Molecular epidemiologic investigation of *Campylobacter coli* in swine production systems, using multilocus sequence typing. *Applied and Environmental Microbiology* 2006; **72**: 5666–5669.
7. **Anon.** Commission Decision of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses to be carried out in the Member States (2007/516/EC). (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:190:0025:0037:EN:PDF). Accessed 5 February 2014.
8. **Moran L, Kelly C, Madden RH.** Factors affecting the recovery of *Campylobacter* spp. from retail packs of raw, fresh chicken using ISO 10272-1:2006. *Letters in Applied Microbiology* 2009; **48**: 628–32.

9. **Vidal AB, *et al.*** Comparison of different sampling strategies and laboratory methods for the detection of *C. jejuni* and *C. coli* from broiler flocks at primary production. *Zoonoses and Public Health* 2013; **60**: 412–425.
10. **Rosenquist H, Bengtsson A, Hansen TB.** A collaborative study on a Nordic standard protocol for detection and enumeration of thermotolerant *Campylobacter* in food (NMKL 119, 3. ed., 2007). *International Journal of Food Microbiology* 2007; **118**: 201–213.
11. **Williams LK, *et al.*** Enrichment culture can bias the isolation of *Campylobacter* subtypes. *Epidemiology and Infection* 2012; **140**: 1227–1235.
12. **Randall L, *et al.*** Development and evaluation of internal amplification controls for use in a real-time duplex PCR assay for detection of *Campylobacter coli* and *Campylobacter jejuni*. *Journal of Medical Microbiology* 2010; **59**: 172–178.
13. **Lawes JR, *et al.*** Investigation of prevalence and risk factors for *Campylobacter* in broiler flocks at slaughter: results from a UK survey. *Epidemiology and Infection* 2012; **140**: 1723–1737.
14. **Gardner IA.** The utility of Bayes' theorem and Bayesian inference in veterinary clinical practise and research. *Australian Veterinary Journal* 2002; **80**: 758–761.
15. **Arnold ME, Cook AJC, Davies RH.** A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of salmonella in pigs. *Journal of the Royal Society Interface* 2005; **2**: 365–372.
16. **Arnold ME, *et al.*** Estimation of the sensitivity of environmental sampling for detection of *Salmonella* Enteriditis in commercial egg-laying flocks relative to the within-flock prevalence. *Epidemiology and Infection* 2010; **138**: 330–339.
17. **Fitzgerald C, Whichard J, Nachamkin I.** Diagnosis and antimicrobial susceptibility of *Campylobacter* species. In: Nachamkin I, Szymanski CM, Blaser MJ, eds. *Campylobacter*. Washington: AMS Press, 2008, pp. 227–243.
18. **Anon.** Microbiology of food and animal feeding stuffs – horizontal method of detection and enumeration of *Campylobacter* spp. Part 1 – detection method. ISO 10272-1, 2006(E).
19. **Rodgers JD, *et al.*** An evaluation of the survival and detection of *Campylobacter jejuni* and *C. coli* in broiler caecal contents using culture based methods. *Journal of Applied Microbiology* 2010; **109**: 1244–1252.
20. **Woldemarium E, *et al.*** The sensitivity and specificity of fecal and cecal culture for detection of *Campylobacter* in Dutch broiler flocks quantified by Bayesian analysis. *International Journal of Food Microbiology* 2008; **121**: 308–312.
21. **Anon.** Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. Part A, *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal* 2010; **8**: 1503.
22. **El-Shibiny A, Connerton PL, Connerton IF.** Campylobacter succession in broiler chickens. *Veterinary Microbiology* 2007; **125**: 323–332.
23. **Best EL, *et al.*** Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiology Letters* 2003; **229**: 237–241.
24. **Leblanc-Maridor M, *et al.*** Rapid identification and quantification of *Campylobacter coli* and *Campylobacter jejuni* by real time PCR in pure cultures and in complex samples. *BMC Microbiology* 2011; **11**: 113.
25. **Choi YK, Johnson WO, Thurmond MC.** Diagnosis using predictive probabilities without cut-offs. *Statistics in Medicine* 2006; **25**: 699–717.