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The sensitivity and specificity of fecal and cecal culture for the detection of *Campylobacter* in Dutch broiler flocks quantified by Bayesian analysis

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Abstract

Dutch broiler flocks are routinely tested for the presence of thermotolerant *Campylobacter* spp. using a standard cultural procedure for fecal and cecal samples. The objective of this study was to estimate the sensitivity and specificity of fecal and cecal culture for detection of *Campylobacter* colonization in broiler flocks in absence of a gold standard. Data from 1600 flocks were used from two different populations, whereby only flocks with both fecal and cecal culture results were included in the analysis. Latent class analysis using Bayesian inference was applied to generate the test characteristics of fecal and cecal culture. Two statistical models assuming conditional dependence of both tests on *Campylobacter* status were used to compare the results. On flock level, the sensitivity of the fecal culture was found to be 21% (95% CI: 12, 31) and 23% (95% CI: 13, 60), and the specificity was 98% (95% CI: 94, 99) and 97% (95% CI: 92, 99) for the two models, respectively. The sensitivity of the cecal culture was 64% (95% CI: 37, 89) and 66% (95% CI: 39, 90), and the specificity was 98% (95 CI: 94, 99) and 95% (95% CI: 72, 99) in respective models. The implications of a low sensitivity as in the case of the fecal culture is important for the design and interpretation of monitoring programmes and may result in excessive false negative test results. Although cecal culture is the more sensitive test, substantial misclassification of infected flocks may still occur.
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Keywords: *Campylobacter*; Cecal culture; Fecal culture; Sensitivity; Specificity; Bayesian inference

1. Introduction

Nowadays thermotolerant *Campylobacter* species, *C. jejuni* and *C. coli*, are the most common cause of acute bacterial gastroenteritis in humans in most developed countries (Wingstrand et al., 2006; Skirrow, 1991; Havelaar, 2004) and the incidence of *Campylobacter* infection has been increasing in many industrialized countries (Lund et al., 2004; Engberg et al., 2001; De Wit et al., 2001). Although rarely fatal, *Campylobacter* infections cause considerable illness and loss of productivity and may be associated with severe disabling consequences, including reactive arthritis and Guillain–Barré Syndrome (Wingstrand et al., 2006; Altekruse et al., 1999).

Thermotolerant *Campylobacter* species live in a wide range of animals, but various studies have shown that poultry and poultry products are the main reservoirs of *Campylobacter* spp. for human infection mainly *C. jejuni* (Jacobs-Reitsma, 1997; Newell and Fearnley, 2003). Several control measures have been implemented to reduce the exposure of humans to *Campylobacter* spp., either by reducing the incidence of *Campylobacter* infections in broiler flocks by biosecurity measures at farms or by improving slaughterhouse hygiene (e.g. Wagenaar et al., 2006). Although proper hygienic measures may reduce the incidence of infections of poultry flocks, it is yet by far not a guarantee that the flock will remain free from *Campylobacter* spp. (Adkin et al., 2006), and consequently cannot rely on as a sole intervention to minimize exposure to humans.

Improvement of intervention strategies that reduce human exposure requires knowledge of the *Campylobacter* status of broiler flocks. Consequently, it is essential to have data on the sensitivity and specificity of tests to detect an infection at flock level. Generally flocks are tested for the presence of *Campylobacter* by culturing fecal and/or cecal samples (Corry et al., 1995; Musgrove et al., 2001; Payne et al., 1999; Berndston

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et al., 1996). Fecal samples are easier to gather, but it is generally assumed that cecal samples are more appropriate, since *Campylobacter* mainly colonizes the cecum and, consequently, fecal samples often contain a lower number of bacteria per gram than cecal samples (Rudi et al., 2004).

The sensitivity and specificity of the two testing procedures have, to our knowledge, never been established. This quantitative information is essential to interpret test results which is, in turn, necessary to develop intervention measures to further reduce human exposure. Preferably, test characteristics are determined by comparing the results with those from a reference test, but perfect reference tests are not available. In absence of the true infection status of animals a Bayesian approach of maximizing the likelihood for the model can be performed (Enøe et al., 2001, Frossling et al., 2003, Orr et al., 2003, Swildens et al., 2005). In this approach, it is assumed that every valid test (a test that performs better than chance) classifies a proportion of the true positive and true negative test-samples correctly (Casella and George, 1992). Moreover, in the Bayesian approach to estimate a parameter, e.g. sensitivity Se, prior information about this parameter Se (from the literature or expert opinion) is used in combination with actual data to obtain a posterior estimate of Se (Branscum et al., 2005).

The aim of this study was to estimate and compare the sensitivity and specificity of fecal and cecal culture for detection of *Campylobacter* species in Dutch broiler flocks using a Bayesian analysis.

2. Materials and methods

2.1. Study population

Since 1997, a monitoring programme for *Campylobacter* colonization of Dutch broiler farms is carried out by The Dutch Product Board of Livestock and Meat (PVE). In this study we used data from 1600 flocks. Out of these 1600 flocks 579 had both fecal and cecal culture results, and belong either to the partial and/or final depletion population (Table 1). About 8% (47) of the flocks have undergone both partial and final depletion. Partial depletion is a process where a small part of early market-weight attaining broilers are slaughtered, followed by final depletion of the remaining flock, usually one week later (Jacobs-Reitsma et al., 2001). Because of the economic benefit, it is practiced by several farmers and slaughter companies. An overview of the technical data is given in Table 2.

Table 1
Observed test results of fecal and cecal *Campylobacter* culture cross-classified into partial and final depletion population

		Cecal culture					
		Partial			Final		
		Positive	Negative	Total	Positive	Negative	Total
Fecal culture	Positive	12	5	17	30	18	48
	Negative	27	57	84	119	311	430
	Total	39	62	101	149	329	478

Table 2
An overview of the technical data of the Dutch broiler flocks tested for *Campylobacter*

	Partial depletion	Final depletion
No flocks	101	478
Mean no. chicken per flock	80,269	78,227
Age (min, max)*	35 (34, 39)	40 (34, 50)
Percent positives**		
Spring	48 (14/29)	6 (9/134)
Summer	70 (19/27)	11 (13/119)
Fall	20 (4/20)	6 (7/107)
Winter	28 (7/25)	4 (5/118)

*Age in days at slaughter.
**Considering a flock positive when the fecal and/or the cecal culture tested positive.

2.2. Collection and processing of samples

Fecal samples (5 per shed) were collected by the farmer a few days before slaughter, put in a sampling bag or tube with identification, and sent to a certified laboratory which was approved by the PVE. The samples were sent by regular mail, generally within 1–2 days after sampling. Cecal samples (30 per batch) were collected at slaughterhouses, and sent to the laboratory immediately. Within 48 h after arrival in the laboratory, samples were examined for the presence of *Campylobacter* by laboratories certified by the PVE (ISO/IEC 17025; www.pve.nl). This includes direct streaking of sampled material on to a *Campylobacter* selective mCCDA plate, followed by micro-aerobic incubation at 41.5 °C for 48 h. According to the PVE protocol, specific colonies are confirmed by oxidase reaction and microscopically. In addition, some samples may be confirmed serologically or by agglutination test (ISO 10272:1995/Cor 1997). The test results were registered in a database at the slaughterhouse.

2.3. Statistical analysis

The values of Se and Sp of the two testing procedures were estimated by Bayesian analysis using the winBUGS program (Spiegelhalter et al., 1996). The test results of fecal and cecal culture may not be independent as they are based on the same biological phenomenon being presence of *Campylobacter* (Gardner et al., 2000). When using a model assuming independence of data, estimates of test accuracy could be misleading if the test outcomes for a given animal appeared to be correlated (Georgiadis et al., 2003), which would result in a biased estimate. Therefore, a model assuming conditional dependence on infection status was used. Such a model would make clear if the data were independent or not.

Two models were used that assume conditional dependence on infection status between tests (Engel et al., 2006 (model 1) and Branscum et al., 2005 (model 2)). The models allow the estimation of the sensitivity and specificity of two tests, based on their cross-classified results, when applied to flocks from two populations with different disease prevalences (Enøe et al., 2001). These parameters have a distribution in stead of a fixed value. The distributions are used to find a combined optimum

for the sensitivity and specificity and the prevalences in that the likelihood for the model is maximal given the data. The parameterization of both models is different. The model of Engel et al. (2006) is based on a normal distribution of the priors, whereas the model of Branscum is based on beta distributions. Posterior distributions were obtained with Markov Chain Monte Carlo (MCMC) methods employing the Gibbs sampler, as implemented in the WinBUGS program.

For the analysis, two populations with a different prevalence of infection had to be made from the data set: one being partially depleted flocks and the second being the finally depleted flocks. Therefore the samples were grouped into those originating from the partial and those originating from final depletion flocks (Table 1). For each population test results are cross-classified in a 2×2 table according to the status of each flock tested (as shown in Table 1). Each 2×2 table provides three degrees of freedom for estimation. When two populations are available, there are 6 d. f. for estimation. The number of parameters of interest is two for each of the two tests (sensitivities and specificities) and one for each of the two populations (prevalences).

The priors of both test procedures indicate that we assumed that the sensitivity of both tests likely would be in excess of 10% (dbeta(1.53, 1.53)) and that the specificity of both fecal and cecal culture was high, i.e. 95% sure that it is greater than 95% (dbeta(88.279, 1.88)) (Wagenaar, pers. comm.). The latter was because growth of bacteria in the selective media has a very low probability of resulting in a positive result. The prior information of the sensitivity and specificity of fecal and cecal culture is shown in Table 3. The prior for the true prevalence of *Campylobacter* in the partially depleted and the finally depleted population was set to an uninformative uniform beta distribution (dbeta(1, 1)) (see also Table 3). For the analyses presented, posterior inferences were based on 50,000 iterations after a burn-in of 5000 iterations being discarded. Convergence was assessed by observing the autocorrelation and by running multiple chains from dispersed starting values and investigating the Brooks–Gelman–Rubin convergence statistic (Gelman and Rubin, 1992). The median of the posterior distribution was used as an estimate for our parameter of interest. The 2.5 and 97.5 percentage points were used for estimating the 95% credibility intervals.

Sensitivity analysis was done by doing separate analyses of the partially and finally depleted population to check if the test characteristics varied across the two populations. In addition, repeated analysis was done for different initial values and priors to see the stability of the output.

3. Results

3.1. Descriptive statistics

3.1.1. Partially depleted population

The total number of flocks in the partially depleted population was 101, and the apparent prevalence of *Campylobacter* in this population based on either fecal and/ or cecal culture positive was 44%. There were 17 (16%) flocks with a positive fecal culture and 39 (38%) flocks with a positive cecal culture (Table 1). The mean age at slaughter of the partial depleted flocks was 35 days, which ranges between 34 and 39 days (Table 2).

3.1.2. Finally depleted population

There were 478 finally depleted flocks, out of which 47 flocks also had undertaken partial depletion. The apparent prevalence of *Campylobacter* considering positive fecal and/or cecal culture as a colonized flock was 35%. There were 48 (10%) flocks tested positive with the fecal culture and 139 (29%) flocks positive with cecal culture (Table 1). The mean age at slaughter was 40 days, which ranges between 34 and 50 days (Table 2).

3.2. Sensitivity and specificity

Model 1 resulted in a sensitivity of 23% (95% CI 13, 60) and a specificity of 97% (95% CI: 92, 99) of the fecal test and a sensitivity of 66% (95% CI 40, 90) and a specificity of 96% (95%CI: 72, 99) of the cecal test (Table 4). The results of model 2 were very similar, a sensitivity of 21% (95% CI 12, 32) and a specificity of 98% (95% CI: 94, 99) of the fecal test and a sensitivity of 64% (95% CI 37, 89) and a specificity of 98% (95%CI: 94, 99) of the cecal test, respectively. Model 1 indicated a true prevalence 60% (95% CI: 37, 96) and 46% (95% CI: 31, 83) for the partial and final depletion population, while model 2 indicated a partial and final depletion population true prevalence of 54% (95% CI: 21, 91) and 40% (95% CI: 11, 72), respectively. The correlation for the sensitivity of the two tests was 0.02 (95% CI: −0.4, 0.24) and for the specificity 0.45

Table 3
Prior probability distributions of the sensitivities and specificities of fecal and cecal culture and the true population prevalence of *Campylobacter* in Dutch broiler

Parameter		Beta distribution	Mode % (95% lower bound)	Source	Convergence
Fecal culture	Sensitivity	dbeta(1.53, 1.53)	50 (10)	It is very low usually with mode 50% (Wagenaar pers. Comm..)	Converged
	Specificity	dbeta(88.279, 1.88)	99 (95)	It is unlikely that there would be false positives (Wagenaar pers. Comm..)	Converged
Cecal culture	Sensitivity	dbeta(1.53, 1.53)	50 (10)	Same as Se fecal culture	Converged
	Specificity	dbeta(88.279,1.88)	99 (95)	Same as Sp fecal culture	Converged
True prevalence	Partial depletion	dbeta(1, 1)		Uninformative	Less converged**
	Final depletion	dbeta(1, 1)		Uninformative	Less converged**

*Convergence was assed by observing the Gelman Rubin statistic and Autocorrelation.
**Less convergence of the two prevalence parameters may be attributed to the uninformative nature of the priors.

Table 4
Estimates and 95% credibility intervals of sensitivity and specificity of fecal and cecal culture and the true prevalence of *Campylobacter* in Dutch broiler flocks estimated by Bayesian inference using two models assuming conditional dependence

		Model 1 (Engel et al., 2006)	Model 2 (Branscum et al., 2005)
	Parameter	% [95% CI]	% [95% CI]
Fecal culture	Sensitivity	23(13, 60)	21(12, 32)
	Specificity	97(92, 99)	98(94, 99)
Cecal culture	Sensitivity	66(40, 90)	64(37, 89)
	Specificity	96(72, 99)	98(94, 99)
Correlation	Sensitivity	4.92(0.007, 68)*	0.02(−0.4, 0.24)
	Specificity	0.02(0.014, 2.3)*	0.45(0.02, 0.9)
True prevalence	Partial depletion	60(37, 96)	54(21, 91)
	Final depletion	46(31, 83)	40(11, 72)

*Percentage increase.

(95% CI: 0.02, 0.9). This means that the tests behave like independent tests for the parameter sensitivity and are slightly correlated with respect to the parameter specificity.

The sensitivity and the specificity remained consistent for both analyses on a partial or final depletion population level, and on a combined level verifying the assumption that test characteristics be the same across the two populations. Further sensitivity analysis was done to see how the priors and initial values influenced the final output. According to expert opinion the sensitivity of *Campylobacter* culture is generally low, and resulted in a prior of 50% (with a 95% interval from 10–90%). To determine a possible effect of a change in estimate, the priors were changed from a mode of 50% and 95% lower limit of 10% dbeta(1.53, 1.53) to a mode of 50% and 95% lower limit of 30% (dbeta(8.00, 8.00)). This implies that not only the lower limit is increased to 30% but also that the upper limit is decreased to 70%. This change of priors did not result in a different outcome.

4. Discussion

The aim of this study was to quantify the sensitivity and specificity of the whole testing procedure including fecal and cecal culture for detection of *Campylobacter* colonization of broiler flocks. Field data were used and two Bayesian models, which assume conditional dependence on infection status, were applied to analyse the data. Both models resulted in comparable estimates for all parameters. In both models it appeared that the sensitivity of cecal culture was much higher than the sensitivity of the fecal culture. The implication of this finding is that it can now be determined how many flocks may have been misclassified as negative. This, in turn, could mean that human exposure to *Campylobacter* might have been much higher than assumed until now. The results can also be used to determine whether and how the test procedure and herd sensitivity could be improved.

The lower sensitivity of the fecal compared to cecal culture could be explained by previous findings that *Campylobacter* mainly colonizes the ceca (Rudi et al., 2004). Another explanation is that fecal samples were collected by the farmer and the interval between sample collection and laboratory testing, and the possible mishandling might have exposed the bacterium to sub-optimal conditions resulting in less culturable bacteria (Havaei et al., 2006). In addition to this, fewer fecal samples are taken than cecal samples, which in general also decreases herd sensitivity (e.g. Dohoo et al., 2003). Concerning the specificity, both the cecal and fecal culture showed high values, because growth of *Campylobacter* in selective culture media is usually considered as unambiguous demonstration of infection.

Sensitivity analysis on partially or finally depleted population and combined population level was done to verify the validity of the assumption that test characteristics were comparable between the two populations. The results showed that the sensitivity and the specificity remained within the same credibility interval. The low correlation between the sensitivity of the two tests can be helpful to generate appropriate estimates of these parameters. Furthermore, the true population prevalence was higher in the partial depletion population than in the final depletion population. This is in contrast to previous studies that demonstrated that *Campylobacter* colonization of a flock increased with time (Hald et al., 2000, 2001; Jacobs-Reitsma et al., 2001; Russa et al., 2005). The high prevalence in partial and the low prevalence in final depletion population might be because about 50% of the partial depletion populations and 90% of the final depletion flocks came from different flocks with distinct prevalence where the former was higher.

Interventions aimed at reducing the likelihood of exposure of consumers to *Campylobacter* directly from poultry and poultry products are expected to contribute to a reduced incidence of illness in humans. Our results may help in improving *Campylobacter* monitoring programmes to reduce the number of false negative flocks. Taking fecal samples has advantages, being easy to collect and less costly. The herd sensitivity of fecal culture could be increased by taking more samples, by improving the handling of samples between collection and laboratory testing (see for example Musgrove et al., 2001). Furthermore *Campylobacter* prevalence reports based on fecal culture of broilers should also consider the low sensitivity of the test, which may underestimate the true value. This is mainly important in risk analysis studies, which make use of prevalence and sensitivity of the test used. Further study could be carried out to get a better estimate with sufficiently large data and with controlled potential extraneous factors such as season and environment and the interval between collection of samples and laboratory testing that may have impact on the culturing process. Then, the surveillance system could be improved and necessary measures to reduce human exposure could possibly be developed.

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