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Quantifying Transmission of *Campylobacter jejuni* in Commercial Broiler Flocks

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ABSTRACT

Since meat from poultry colonized with *Campylobacter* spp. is a major source for bacterial gastro-enteritis, human exposure should be reduced by, amongst others, prevention of colonization of broiler flocks. To gain more insight into possible sources of introduction of *Campylobacter* into broiler flocks, it is essential to have estimates of the moment that the first bird in a flock becomes colonized. If the rate of transmission within a flock was known, such an estimate could be determined from the change in prevalence of colonized birds in a flock over time.

The aim of this study was to quantify the transmission rate of *Campylobacter* using field data gathered during 5 years in Australian broiler flocks. We used unique sampling data from 42 *Campylobacter jejuni* colonized flocks and estimated the transmission rate parameter β, which is defined as the number of secondary infections caused by one colonized bird per day. The estimate was 2.37 ± 0.295 per infectious bird / day, which implies that in our study-population colonized flocks of 20,000 broilers would show an increase of within-flock prevalence to 95%, within 4.4 to 7.2 days after colonization of the first broiler. Using Bayesian analysis, the moment of colonization of the first bird in a flock was estimated to be from 21 days of age onwards in all flocks in the study.

This study provides an important quantitative estimate of the transmission rate of *Campylobacter* in broiler flocks, which could be helpful in future studies on the epidemiology of *Campylobacter* in the field.

INTRODUCTION

Campylobacter spp. are a common cause of diarrhea in humans, and many cases of campylobacteriosis are associated with the handling and consumption of contaminated poultry

meat (5). Various studies on the epidemiology of *Campylobacter* have resulted in the implementation of biosecurity and hygienic measures on poultry farms and slaughterhouses with the ultimate goal to reduce human exposure (6, 8). These measures, most likely, contributed to a reduction of the number of *Campylobacter*-positive broiler flocks. Nevertheless, contaminated meat is still on the market (11), and a further reduction of the flock prevalence is considered necessary by public health authorities in many countries (4). Clearly, more knowledge on the mechanism of introduction of *Campylobacter* into a flock is essential to improve the current control programs. This would, in turn, require an estimate of the moment a flock becomes colonized. It seems, however, unfeasible to detect the first bird that becomes colonized in commercial broiler flock, because of flock size and necessary sampling frequency.

An alternative approach is to determine the transmission rate of *Campylobacter* within a flock. The transmission rate parameter β, which is defined as the number of secondary infections caused by one colonized bird per day, determines the rate of increase in the number of colonized birds over time. It can be used to determine the moment of introduction from field data on increasing *Campylobacter* prevalence over time. Estimates for the parameter β have been provided in experimental studies (16, 17) being 1.04 - 1.13 per day. Experimental conditions differ, however, substantially from the field situation, which implies that the transmission rate should also be estimated in commercial flocks.

A series of field studies in Australia were carried out between 1999 and 2004 in which broiler flocks were sampled with daily to weekly frequency. The aim of these studies was to develop an understanding of the epidemiology of *Campylobacter* in Australian broiler flocks. We analyzed data from this unique dataset to estimate the *Campylobacter* transmission rate in commercial broiler flocks. Additionally, we estimated the moment the first bird in a flock became colonized with *Campylobacter* (for reasons of convenience we refer to this event as the

56 moment of introduction of *Campylobacter* in a flock) and assessed how accurately these

57 moments of introduction can be estimated.

MATERIALS AND METHODS

Dataset

Three longitudinal studies were carried out in South East Queensland, Australia, between 1999 and 2004. A subset of the full dataset was selected for the current study: for flocks to be included in the current analyses, they should have been sampled at least twice, with at least one *Campylobacter* positive dropping detected. Flocks with migration barriers separating groups of birds within a shed, and flocks with missing sampling age were excluded. A total of 42 flocks met all the inclusion criteria (see supplementary material for details).

At each sampling age randomly selected individual fecal or cecal droppings were collected immediately after the droppings were produced. Birds that produced these droppings were not marked, as the likelihood of sampling a single bird more than once a day was considered limited in these large populations. Care was taken to ensure that the sample was collected without any contaminating material. The samples were collected with a sterile swab which was immediately placed in a sterile container. Samples were held on ice during transport to the laboratory, and were streaked on Karmali *Campylobacter* Agar Base (Oxoid CM935, Oxoid, Melbourne) containing *Campylobacter* Selective Supplement (Oxoid SR167E) immediately after arrival in the laboratory. Agar plates were incubated at 42^{o}C for 48 hours in a tri-gas incubator with an atmosphere of 85% N_2, 10% CO_2 and 5% O_2. Colony morphology and cell motility under phase contrast microscopy were used to confirm identification to genus level. Single colonies from a number of positive samples from each positive flock were subcultured onto Sheep Blood Agar and incubated as mentioned above before being identified to species level by tests for oxidase, catalase production, and hippurate hydrolysis.

The final data consisted of records of sampling events for each flock, with age of the flock t, sample size n_t, flock size at the start of the rearing period N, and number of positive samples x_t (see supplementary material for details).

Modeling of *Campylobacter* transmission in broiler flocks

It is generally assumed that, following the onset of *Campylobacter* colonization of the gut, broilers shed the bacteria lifelong (1). Therefore, we assumed that birds are either susceptible (non-colonized) or infectious (colonized), and that increase in prevalence can be described by a susceptible-infectious (*SI*) type mathematical model (13, 17). In this model, susceptible chicks can be colonized upon contact with an infectious chick, which occurs with rate $\beta i(t)$, a product of the proportion of infectious chicks in the shed $i(t)$, and transmission rate β. The transmission rate is interpreted as the mean number of chickens that can be colonized by one infectious chicken per day, in a susceptible population.

In the large populations of broilers usually present in commercial flocks, the change of the proportion of infectious birds $i(t)$ can be approximated by the deterministic differential equation:

$$\frac{di(t)}{dt} = \beta(1 - i(t))i(t), \quad (1)$$

of which the solution is the logistic curve

$$i(t) = \frac{e^{\beta(t-\tau)}}{1 + e^{\beta(t-\tau)}}, \quad (2)$$

in which τ is the time at which 50% of the birds is infected. From τ, β and flock size N,

$$t_0 = \tau - \log(N-1)/\beta \quad (3)$$

is calculated as the time when transmission starts.

Estimation of β in the study population

Because the increase in the proportion of colonized birds follows a logistic curve, the transmission rate β was estimated by a logistic regression analysis of the numbers of positive

samples x_t in time. This is only possible, however, if in at least two samples only part of the swabs are is positive, because only then the steepness of the increase in prevalence (determined by β) is observed. Eight flocks met this criterion. The *SI* model was fitted for each of these eight flocks by a logistic regression of the binomially distributed x_t (and sample size n_t) with t as covariate, and $\exp(\tau)$ as intercept. The model fits resulted in eight estimates for β and τ, from which t_0 can be estimated by use of equation (3), N being the flock size at the start of the rearing period. Confidence intervals for β and t_0 were derived by profile-likelihood (3), which was carried out in Mathematica (version 6.0; Wolfram Research, Inc. [http://www.wolfram.com]).

Because flocks with low β have been more likely to meet the inclusion criteria for the logistic regression analysis than flocks with high β, bias would be introduced if the eight estimates were considered representative for all commercial broiler flocks. Therefore, in a next step, we estimated the mean μ_β and standard deviation σ_β of a normal distribution of β among all flocks by maximizing the likelihood function

$$\ell(\mu_\beta, \sigma_\beta) = \prod_{flocks}\left[\int_0^\infty p(\beta)\prod_t i(t)^{x_t}\left(1-i(t)\right)^{n_t-x_t} d\beta\right],$$

in which $p(\beta)$ is the density of the normal distribution of β, $i(t)$ is the logistic curve of equation (2), and t, x_t, and n_t are the data for all flocks. This maximum likelihood estimation method does not result in any flock specific estimates of β or t_0; estimation results are limited to μ_β and σ_β, with confidence intervals (95%) derived by profile-likelihood (3). Although β cannot be negative, the assumption of the normal distribution is valid, because the distribution lies well above zero (see results). The analysis was carried out in Mathematica.

Estimation of the moment of introduction (t_0) in the study population

Because no flock-specific estimates of t_0 could be obtained with the above analysis, we used Markov Chain Monte Carlo integration to obtain Bayesian posterior distributions of β and τ, and therefore t_0, for each flock separately (7). The prior distribution of τ was uninformative (flat), whereas the prior distribution of β was the normal distribution resulting from the maximum likelihood (ML) estimation (with $\mu_\beta = 2.37$ day^{-1} and $\sigma_\beta = 0.295$). The likelihood function was $\ell(\beta,\tau) = \prod_t i(t)^{x_t}\left(1-i(t)\right)^{n_t-x_t}$.

We used the ML result from all 42 flocks as input for the estimation of t_0 in the same flocks, where it would have been more correct to divide the data into two mutually exclusive subsets, and use both subsets only once. However, the current approach results in a more precise estimate of μ_β and σ_β , and estimates of t_0 for all flocks instead of only a subset. Possible errors resulting from our approach are minor, as observed from a separate sensitivity analysis of estimation of t_0 to correctness of the prior distribution of β (see below).

The posterior distribution was sampled 10,000 times by single-component Metropolis-Hastings sampling, after 100 samples for burn-in (9). The mean of the (normal) proposal distributions for the (i + 1)st samples of both β and τ were the ith samples; the standard deviation of the proposal distribution of β was 0.295; the standard deviation of the proposal of τ was determined from the dataset: it was one quarter of the time interval between the last 0% prevalence sample and first 100% prevalence sample. This resulted in means and 95% credible intervals of t_0 for 40 of the 42 flocks (for two flocks, flock size was not known). The sampling algorithm was programmed in Mathematica.

Accuracy of t_0 estimation

Although the Bayesian estimation of t_0 does provide 95% credible intervals, these are based on the logistic curve which does not take account of the stochastic nature of transmission in the

early phase of a *Campylobacter* outbreak. Therefore, we assessed accuracy of the method by estimating t_0 from simulated outbreaks in which the estimates can be compared to the real value.

We simulated 10,000 outbreaks in flocks of 20,000 chicks with values for β sampled from the estimated distribution (μ_β: 2.37 day^{-1}; σ_β: 0.295), with introduction time $t_0 = 0$. Simulations were carried out as described (17), with three sample sizes (10, 20, 100 birds) and three sampling frequencies (once every day, every 3rd or 7th day). This resulted in 10,000 simulated datasets for all combinations of sample sizes and sample frequencies, which were subsequently analyzed by the Bayesian method described above, to obtain posterior means of 1000 samples of the posterior distribution.

In addition to these 10,000 simulations, where the population distribution of β (used in the simulations) was identical to the prior distribution in the Bayesian analysis, we assessed accuracy by simulation with other distributions, based on the confidence intervals for μ_β (2.19-2.58 day^{-1}) and σ_β (0.144-0.488). Four new datasets were simulated, two with different μ_β (2.19 and 2.58 day^{-1}, keeping σ_β at 0.295), and two with different σ_β (0.144 and 0.488, keeping μ_β at 2.37 day^{-1}). This resulted in four sets of 1000 simulated datasets for 10 samples every 1, 3, or 7 days. These were used to estimate t_0 as described above.

RESULTS

Dataset

The flock age at first detection of *Campylobacter* positive droppings varied from 24 to 54 days of age. In 14 out of 42 flocks prevalence was over 95% at first detection. For an additional 10 flocks with prevalence up to 10% at first detection, sampling continued till they were found fully colonized (>95% prevalence), which took 4.4 days on average with a range from 3 to 6 days (see supplementary material for details).

In 38 out of 42 flocks, all *Campylobacter* isolates were typed as *C. jejuni*. In four flocks both *C. jejuni* and *C. coli* were found. Because insufficient data on *C. coli* outbreaks were available, analyses were solely based on *C. jejuni* outbreaks.

Estimation of β and t_0 in the study-population

For eight flocks with sufficient data, flock-specific β and t_0 were estimated by logistic regression. The point estimates of β ranged from 1.3 to 3.1 (Figure 1), and t_0 ranged from 21 to 35 days (see supplementary material for details).

The estimates for the distribution of β, which is based on 42 outbreaks, were: $\mu_\beta = 2.37$ day^{-1} (CI: 2.19 - 2.58) and $\sigma_\beta = 0.295$ (CI: 0.144 - 0.488) (Figure 1), with μ_β and σ_β representing the mean and standard deviation of β. Figure 2 shows how fast flocks of 20,000 broilers were colonized for the mean, a low, and a high transmission rate within the distribution of β.

For 40 *Campylobacter* outbreaks with known population size we estimated t_0 by Bayesian analysis. The results (Figure 3) show that colonization had not taken place before chickens had reached the age of three weeks. On average the moment of introduction t_0 was 4.8 days (range 2.2 to 9.3) earlier than first detection of *Campylobacter* positive samples (see supplementary material for details).

The estimates by logistic regression and Bayesian analysis did not differ more than 0.6 day, except for one flock (3.4 days difference).

Accuracy of t_0 estimation

Accuracy of the Bayesian method was assessed by analyzing simulated outbreaks; the results are shown in Table 1. If the prior distribution used for the analysis reflects the underlying transmission rates in the population correctly, the moment of introduction was estimated within

a margin of 3 days even with weekly sampling of only 10 birds.

The Bayesian estimation method was not very sensitive to the correctness of the prior distribution (within the margins of the 95% confidence intervals), sensitivity being highest if μ_β would be lower or σ_β would be higher, resulting in a margin of 3.3 days with weekly sampling of 10 birds.

DISCUSSION

The first aim of this study was to quantify the transmission rate of *Campylobacter* in commercial broiler flocks. The parameter β, based on the field data, was estimated to be 2.37 day^{-1} (± 0.295), which means that one colonized bird can, on average, infect 2.37 birds per day. This implies that in a flock of 20,000 broilers the within-flock prevalence of *Campylobacter* would increase from a prevalence of one colonized bird to a prevalence of 95% within one week.

Previous studies showed that the flocks detected *Campylobacter* negative at one moment, can appear fully colonized within one week, suggesting an incursion in the intervening week (2, 10, 12). This seems consistent with our findings, but the sample size applied in these studies did not allow for detection of positive flocks in the starting phase of an epidemic. This implies that an incursion could have occurred longer before.

The second aim of this study was to estimate the moment of colonization of the first bird in a flock (t_0), by using this parameter estimate β.

The estimates of t_0 were all above the flock age of 21 days. The estimate of t_0 was accurate by a margin of three days in 90% of the cases, even if only ten birds are sampled weekly. Sensitivity to incorrectness of the prior distribution was low. The apparent lack of *Campylobacter* transmission in the first three weeks of life, as seemed to have occurred in our study has been described before (12). It is difficult or probably impossible to determine whether in the first

three weeks no introduction has occurred or whether it had but had not resulted in colonization of the birds. Risk of introduction of *Campylobacter* can be high throughout the rearing period, but it could also be reasoned that it may increase over time. Another possibility is that young chicks are less susceptible as indicated by Ringoir et al. (14) and Sahin et al. (15), who demonstrated an effect of age and maternally derived immunity on susceptibility. More insight in the underlying mechanism of this phenomenon may provide clues for *Campylobacter* prevention.

CONCLUSIONS

A unique data set describing the change in prevalence of *Campylobacter* colonized birds in commercial broiler flocks was used to quantify *C. jejuni* transmission under field conditions. The estimated transmission rate implies that in a flock of 20,000 broilers the within-flock prevalence of *Campylobacter* increases to 95% within a week after colonization of the first bird. Since such rapid spread has not been described from previous experimental transmission experiments, this study provides important new quantitative information on the epidemiology of *Campylobacter* in broilers.

The study also shows how the transmission rates can be used to estimate when the first bird becomes colonized. We showed that this estimation method was accurate, and therefore this method is promising for further studies into mechanisms of *Campylobacter* introduction, because it allows focusing on the chronology of events.

Additionally, t_0 estimation can result in an accurate description of the period in which no transmission of *Campylobacter* occurs, like the first three weeks in our study population. Consequently, interventions aimed at prevention of introduction of and subsequent colonization with *Campylobacter* might better be targeted at the second half of the rearing period, which, in our study- population, could be considered a high risk period.

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308 Figure 1. Top: the eight estimates of *Campylobacter* transmission parameter β (95%

309 confidence interval), estimated by logistic regression. Bottom: estimated density of the normal

310 distribution of β in the Australian study population, acquired by maximum likelihood

311 estimation: $\mu_\beta = 2.37$ day^{-1}, $\sigma_\beta = 0.295$ day^{-1}.

312 <<Here: figure 1 should be inserted>>

313

314 Figure 2.

315 Epidemic curves of three different values of the estimated distribution of β (lower limit, point

316 estimate, upper limit) and an experimentally derived β estimate (17) in flocks of 20,000

317 broilers.

318 <<Here: figure 2 should be inserted>>

319

320 Figure 3.

321 Histogram of the moments of introduction of 40 Australian *Campylobacter jejuni* outbreaks.

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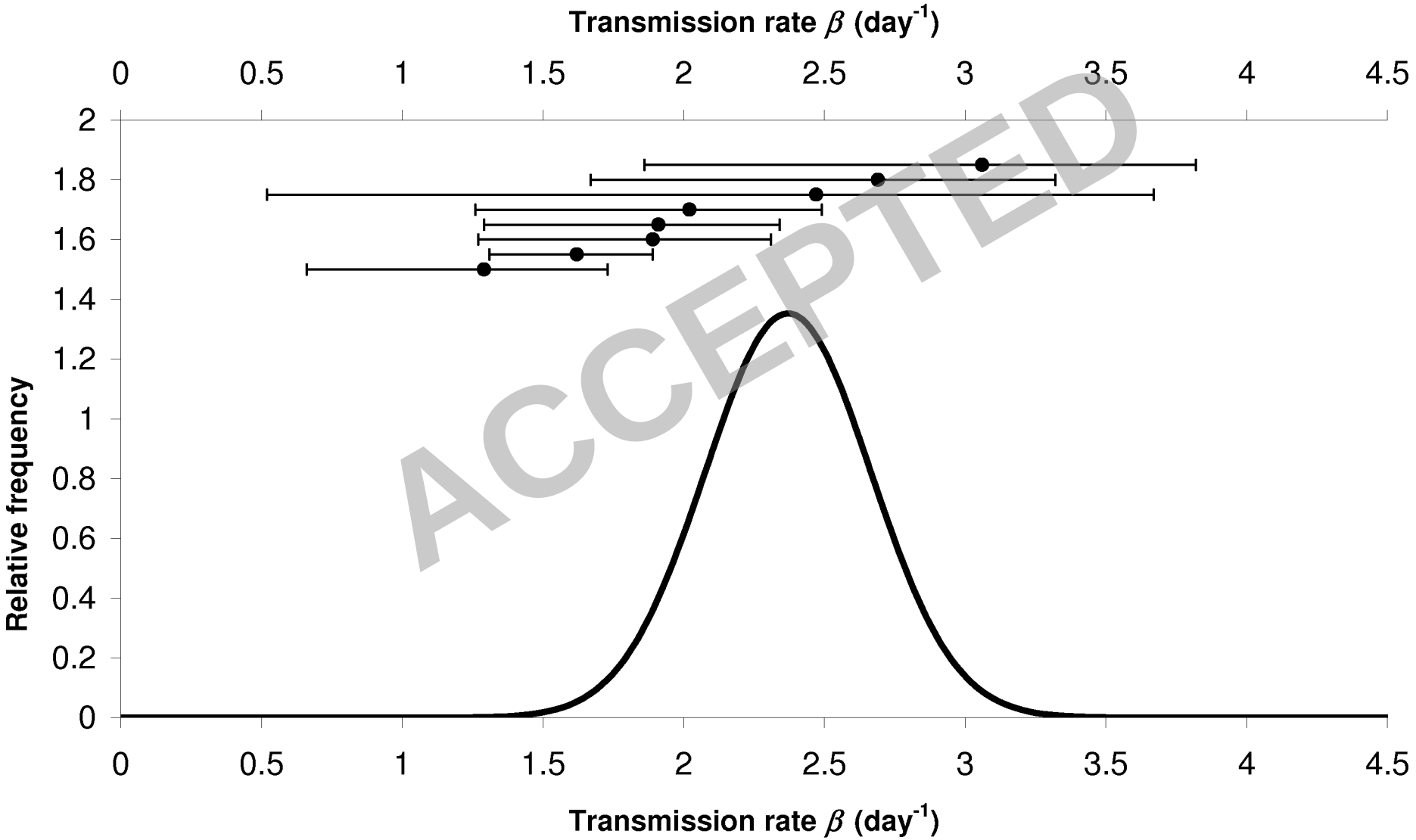
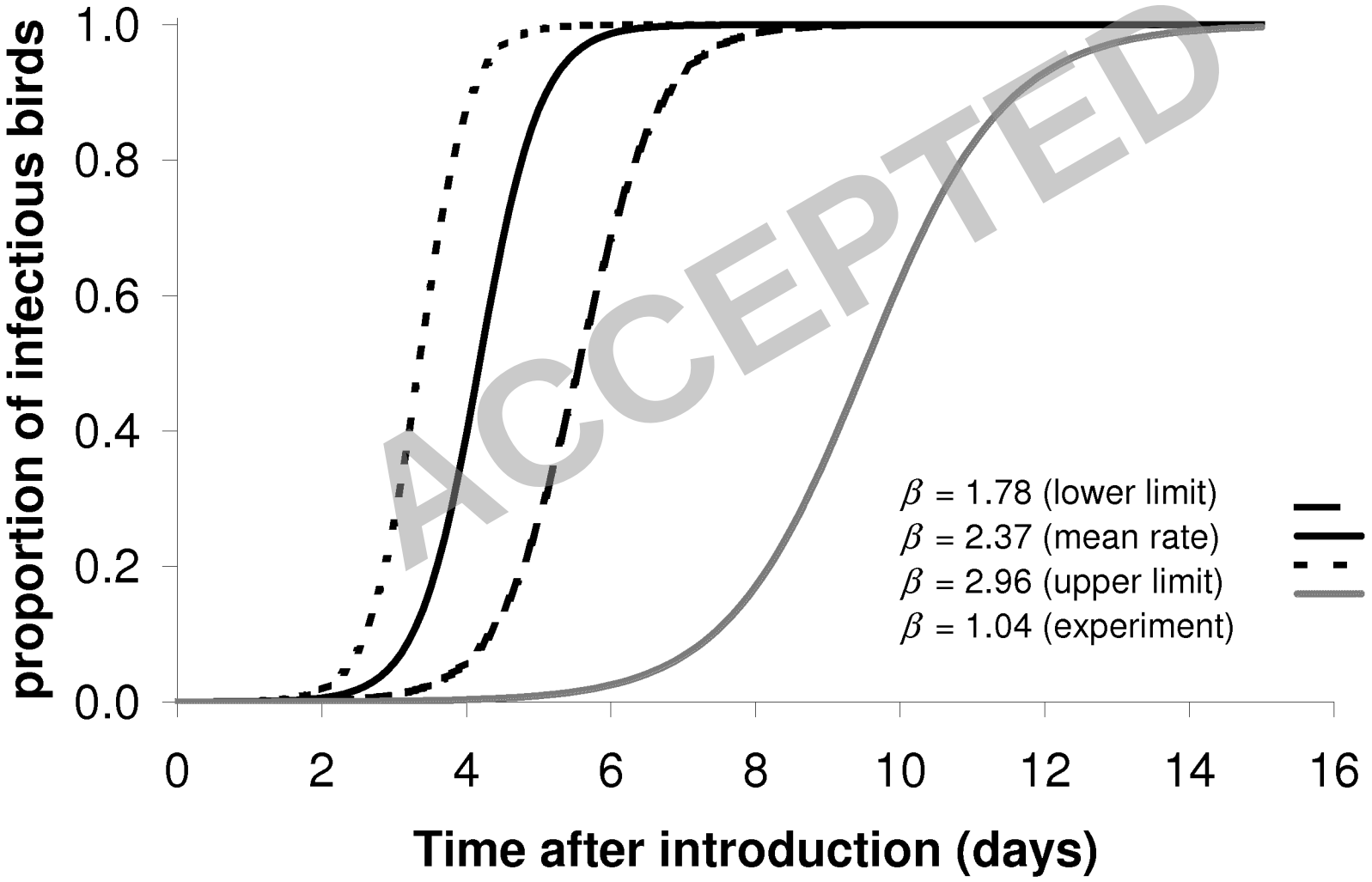
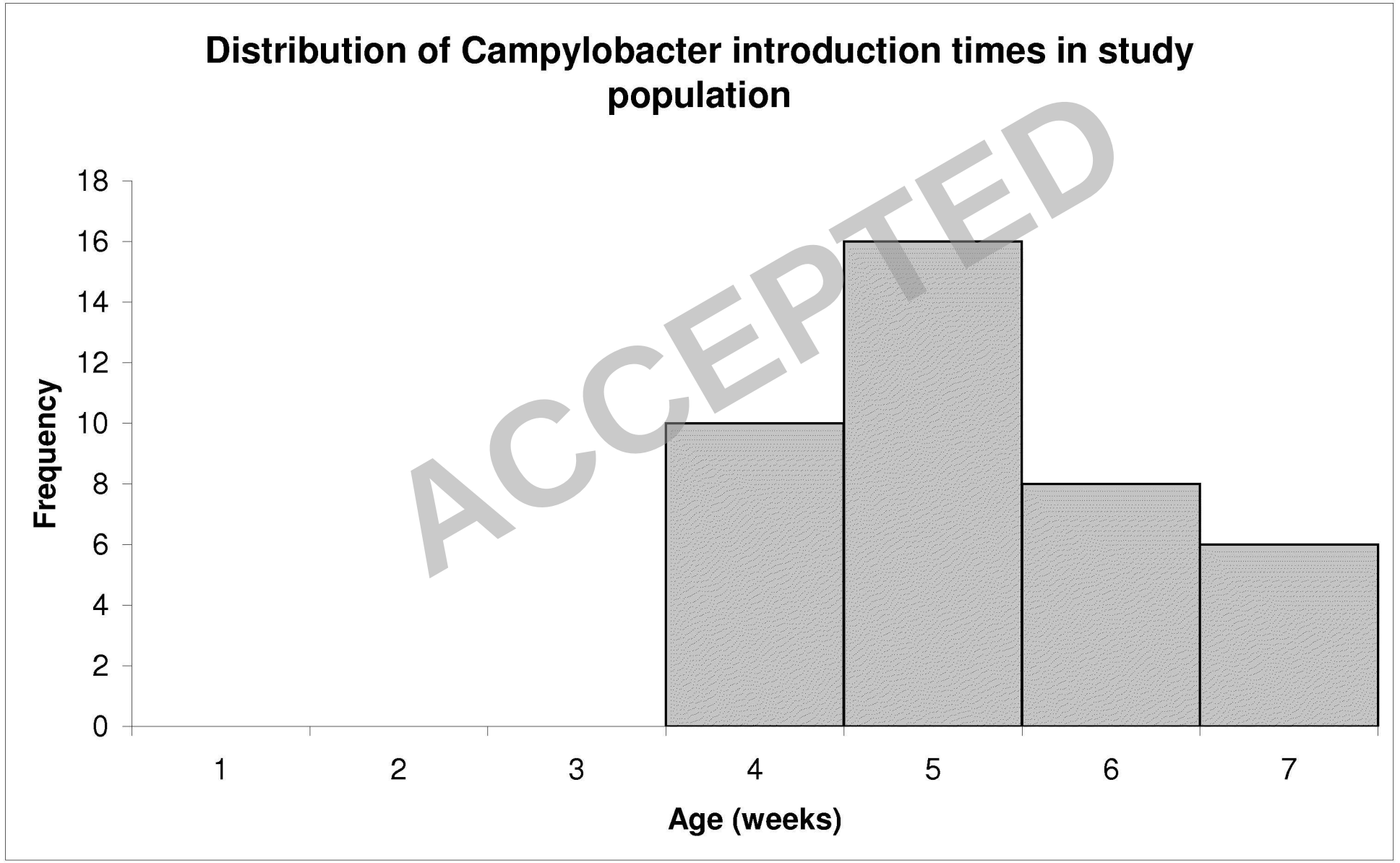
Table 1. Accuracy of Bayesian estimation (with prior distribution μ_β: 2.37 day^{-1} and σ_β: 0.295) of the posterior mean time of *Campylobacter* introduction into a flock of 20,000 broilers, with different sample sizes and sampling intervals. The top rows summarize results from 10,000 datasets simulated with the point estimates of μ_β = 2.37 day^{-1} and σ_β = 0.295. The bottom four rows summarize results from 1000 datasets simulated with different distributions as indicated.

Parameters dataset		sample size	90% interval of estimation error		
μ_β	σ_β		each day	each 3rd day	each 7th day
2.37	0.295	10	-0.9; 1.7	-1.0; 1.8	-2.1; 2.7
		20	-0.8; 1.6	-0.9; 1.7	-1.8; 2.4
		100	-0.6; 1.4	-0.8; 1.6	-1.3; 2.1
2.19	0.295	10	-0.7; 2.2	-0.8; 2.4	-1.7; 3.1
2.58	0.295	10	-1.1; 1.3	-1.4; 1.3	-2.5; 2.3
2.37	**0.144**	10	-0.8; 1.4	-0.8; 1.4	-1.9; 2.5
2.37	**0.488**	10	-1.0; 2.3	-1.3; 2.7	-2.4; 3.3


Transmission rate β (day^{-1})
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Relative frequency
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Transmission rate β (day^{-1})


proportion of infectious birds
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
Time after introduction (days)
β = 1.78 (lower limit)
β = 2.37 (mean rate)
β = 2.96 (upper limit)
β = 1.04 (experiment)


Distribution of Campylobacter introduction times in study population
Frequency
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
Age (weeks)