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Bayesian statistics for infection experiments

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Abstract

To intervene cycles of food-borne pathogens in poultry new intervention methods need to be tested for their effectiveness. In this paper a statistical method is described that was applied to quantify the observed differences between test groups and control groups.

Treated chickens and their controls were inoculated with several doses and were daily examined for the shedding of the tested pathogens.

For these infection experiments with individually housed chickens and where binary data were available for each individual chicken a Bayesian analysis employing Markov Chain Monte Carlo (MCMC) was applied for the statistical analyses. The Cox' proportional hazard reflected the typical features of the data, i. e. dependency, waiting-time structure and censoring. The outcomes of the analyses are two measures of difference in susceptibility between the feed groups. The first effect measure is a relative risk of being infected. The second is a difference in waiting time or a difference in inoculation dose to get a comparable proportion of infected animals.

Keywords: survival analyses; Markov Chain Monte Carlo; infection experiments; susceptibility; epidemiology

Introduction

Salmonella and *Campylobacter* are food-borne pathogens that frequently cause moderate to severe gastroenteritis in humans. Poultry meat is an important source of infections for this human disease. Before poultry feed becomes poultry meat, several steps are run through. A schematic picture of these steps in the poultry production chain is shown in Figure 1. It illustrates that the broiler farm is a crucial link in this chain. This is due to growth of the bacteria in the chickens on the farm and their transmission towards susceptible chickens in the flock. So both the number of bacteria and the number of infected chickens can increase. When chickens become infected with *Campylobacter* or *Salmonella* at the farm, they can cause contamination of equipment and meat products in the subsequent production steps.

The epidemiology of infection in chicken flocks has two typical features. The first is the introduction into the flock. The second is the transmission of infection from infected chickens to susceptible flock mates. Therefore it is necessary to prevent both

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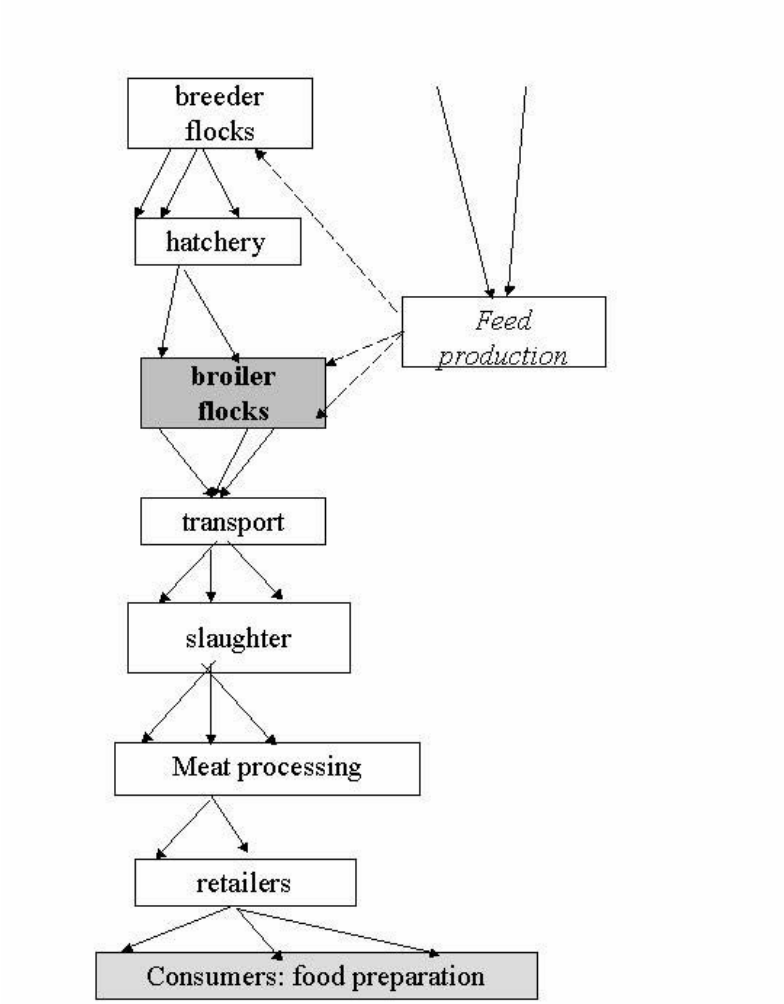

Figure 1. Schematic figure of the poultry production chain, which stresses partitioning and mixing of animals and products

introduction and transmission in order to prevent the multiplication of these pathogens in the broiler flock.

A prerequisite for all effective intervention strategies is the prevention of introduction by strict hygiene. But as these hygiene measures easily fail, additional interventions are necessary to combat the food-borne pathogens effectively. These additional interventions (vaccination (Zhang Barber, Turner and Barrow 1999), medication and competitive exclusion (Impey and Mead 1989)) should make chickens less susceptible or less infectious. If chickens are less susceptible, introduction into the flock is more difficult, and also transmission might be hampered. If chickens are less infectious transmission will be reduced.

The probability that introduction into a flock occurs is best reflected by quantitative estimates of susceptibility for infection of individual chickens. The transmission between chickens can be quantified by comparison of the course of infection in groups of chickens with inoculated chickens and susceptible contact chickens.

In this paper a statistical method will be described that was applied to quantify the changes in susceptibility of treated chickens. This statistical method was applied for experiments with fermented liquid feed (FLF), which was tested for its role in the control of *Salmonella* and *Campylobacter* in broiler chickens. This innovative feed was

expected to reduce the susceptibility for infection and subsequently the transmission of the pathogens between chickens. To test the effect on susceptibility, inoculation experiments were performed. Results of these experiments were described previously (Heres et al. 2003a; 2003b; 2003c). To quantify the effect of intervention on transmission, separate transmission experiments were performed (Heres et al. 2003b), but the analyses of these results lay beyond the scope of this paper.

Materials and methods

The treatment in the experiments described in this paper was fermented liquid feed. This liquid feed was fermented by adding *Lactobacillus plantarum*. The feed:water ratio was 1:1,4. More details can be found in previous papers (Heres et al. 2003a; 2003b; 2003c), but lay beyond the scope of this paper.

The treatment or the control feed was fed throughout the experiment to Ross broiler chickens. The chickens were housed individually after inoculation at one week of age. Various inoculation doses (10^3, 10^4, 10^5, 10^6, 10^7, and even 10^8 cfu/ml) were applied per experiment. Per dose and feed group 10 chickens were inoculated. To detect shedding by bacteriological culture, a faeces sample of each individual chicken was collected every day with a cloacal swab. The outcome of this test was either negative (no shedding) or positive. Parts of the data are shown in Table 1, as an example.

Table 1. Example of data from inoculation experiments with *Campylobacter*

		sample results — days after inoculation —												
feed group	dose (in ^{10}log cfu)	1	2	3	4	5	6	7	8	9	10	11	12	13
1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	0	0	0	0	0	0	1	1	1	1	1	1	1
1	3	0	0	0	0	0	0	0	0	0	0	1	1	1
….														
1	7	0	0	1	1	1	1	1	1	1	1	1	1	1
1	7	0	1	1	1	1	1	1	1	1	1	1	1	1
….														
0	3	0	1	1	1	1	1	1	1	1	1	1	1	1
0	3	0	0	0	0	1	1	1	1	1	1	1	1	1
…..														
0	7	1	1	1	1	1	1	1	1	1	1	1	1	1
0	7	1	1	1	1	1	1	1	1	1	1	1	1	1
…..														

There are three typical features of the data. First, there is dependence between binary observations of the same animal. Second, the structure is typical for a waiting-time problem: i.e. waiting-time until an animal starts shedding the pathogen of study. And finally, some data are censored. A proper model for these data should reflect these typical features. Therefore survival analysis was chosen to calculate the significance of observed differences, i.e. a Cox' proportional-hazards model.

In this model it is assumed that the hazard of getting infected for the treated versus the control group is constant in time:

$$\frac{\text{risk of getting the infection for treated animals}}{\text{risk of getting the infection for control animals}} = \text{Relative Risk} = \text{RR}$$

log RR = linear combination of effects of treatment and dose
$= \beta_1 X_1 + \beta_2 X_2 + \ldots = \beta \text{ ' } X = \eta.$

The hazard function is $h(t) = h_0(t) \exp(\eta)$, with baseline hazard function $h_0(t) = rt^{r-1}$. The r is a shape parameter.

The statistical analyses were performed as follows. For each interval (t_1,t_2) between successive time points t_1 and t_2 where observations are collected, we can calculate the conditional probability *p* that an animal becomes positive in this interval given that it was negative up to time t_1:

$$p = (S(t_1) - S(t_2)) / S(t_1),$$

where S(t) is the survivor function. The product of these probabilities is the likelihood function evaluated for the binary data at the observation times.

An algorithm was developed, based on iterated regression, to calculate maximum likelihood (ML) estimates (Cox and Hinkley 1974) for the treatment and dose effects in RR and for parameter r of the base line hazard. The programme was written in the statistical programming language GenStat (Payne 2000). Also an analysis was performed based on Markov Chain Monte Carlo (Gelman et al. 1995) with the BUGS program (Spiegelhalter et al. 2003). Non-informative priors were chosen for treatment and dose effects in β (normal distributions with mean 0 and variance 10,000) and for the positive parameter *r* in the baseline hazard (gamma distribution with mean 1,000 and variance 1,000,000). Results for ML and Gibss sampling were quite similar for r and β (in the Bayesian analysis the medians of generated parameter values were used as point estimates and Bayesian intervals were considered approximate confidence intervals). Because for a variety of models the analysis is much easier to perform with BUGS than with ML, we proceeded with the Bayesian analysis, which was regarded as an approximate ML analysis.

Models were fitted where η was a linear function of dose (in ^{10}log cfu) with the intercept *a* depending on the treatment, but a common coefficient *b* for the dose (in ^{10}log cfu). To test for lack of fit, models with quadratic functions of dose (in ^{10}log cfu) ($c*dose^2$) were fitted as well. When b (and c) differed significantly from 0, there was a significant relationship between the shedding of the pathogen and the inoculation dose.

Since Δa is the difference between the constants of the FLF group and the control group, factor $e^{\Delta a}$ represented an increase (or decrease) of the probability to start shedding the pathogen of the FLF group compared with the control group in a short time interval after time t, given that the animal did not shed *Campylobacter* up to time t.

Median waiting times *m* were calculated:

$$m = (\log(2) \exp(-\eta))^{1/r}$$

Plots of m against dose (in ^{10}log cfu) enabled us to estimate the concentration needed such that on average half of the animals will shed *Salmonella* at a chosen time. Or alternatively, the time we have to wait before half of the animals will shed *Salmonella* for a chosen concentration. Both η and r are important parameters in the median waiting-time m; extreme and positive values of η correspond to short median waiting-times and extreme and negative values of η to long waiting-times until shedding of *Campylobacter*.

In some of the experiments a probability *se* was included that an animal which sheds *Salmonella* or *Campylobacter* is positive according to the test. The extra parameter *se* was referred to as the sensitivity of the test. The *se* was estimated with a homogeneous prior distribution on the interval <0;1>. Values close to one indicate a high sensitivity. In view of the moderate size of our data sets more complex models were not considered

Results

The results of this method will be illustrated with the data of two experiments with *Campylobacter* that were described previously (Heres et al. 2003b). The percentage of infected chickens is summarized in Table 2, per experiment, per feed group at the different days of sampling. The data suggest that FLF-fed chickens are less susceptible for an infection with *Campylobacter*. FLF-fed chickens appear to need an inoculation dose that is 1 to 2 log cfu higher to infect a comparable proportion of chickens.

Table 2. *Campylobacter*-positive cloacal swabs and final number of CFU in caecum of broiler chickens fed with dry feed and fermented liquid feed that were inoculated with different doses of *Campylobacter* in experiment A and B

		Experiment A				**Experiment B**					
		Campylobacter dose (^{10}log CFU/ml) [1]									
		5.5		**8.6**		**3.0**		**4.0**		**5.0**	
	feed group [2]	**FLF**	**DF**	**FLF**	**DF**	**FLF**	**DF**	**FLF**	**DF**	**FLF**	**DF**
	chickens(n)	*n=10*	*n=10*	*n=10*	*n=10*	*n=10*	*n=10*	*n=10*	*n=10*	*n=8*	*n=8*
		Positive swabs (%)									
days after infection	**1**	0	60	30	90	0	0	0	0	0	75
	2	30	100	80	100	0	10	0	20	25	88
	3	70	100	100	100	0	70	10	90	38	100
	4	90	100	100	100	0	70	30	100	88	100
	5-8	100	100	100	100	0	70	40	100	100	100
	9-13	-	-	-	-	0	70	50	100	100	100

[1] *Chickens were inoculated with 0.25 ml of the suspension;*
[2] *DF= dry feed, FLF = fermented liquid feed*

Parameter estimates from the Bayesian analysis are shown in Table 3.

Table 3. Parameter estimates from statistical analyses of data from different (sets of) experiments

	experiments A and B	
parameter	**posterior median**	**95% interval**
a	-0.67	<-1.1 ; -0.1>
Δa	-2.5	<-3.6 ; -1.8> *
b	1.2	<0.91 ; 1.6> *
c	-0.23	<-0.36; -0.13> *
r	1.5	<1.2 ; 1.9 >
se	0.98	<0.96 ; 0.99>

Posterior medians are taken as point estimates; Bayesian intervals are taken as 95% confidence intervals.
The linear predictor $\eta = \beta' X = aX_1 + \Delta a\, X_2 * + bX_3 + c * X_4$, where $X_1 = 1$, $X_2 = -0.5$ for the control group and 0.5 for the FLF group, $X_3 =$ dose-5.22 and $X_4 = (\text{dose}-5.22)^2$.
a = mean constant at dose level 5.22, Δa = difference between constants of FLF group and control group. $e^{\Delta a}$ represents an increase (or decrease) of the probability to start shedding *Campylobacter* of the FLF group compared with the control group in a short time interval after time t, given that the animal did not shed *Campylobacter* upto time t. Large negative values indicate that animals tend to start shedding *Campylobacter* at a much later stage in the FLF group compared with the control group.
b, c = coefficients for linear and quadratic terms for dose. These coefficients describe the relationship with the *Campylobacter* dose; when they differ significantly from 0 there is a significant relationship between the shedding of *Campylobacter* and the inoculation dose.
se = sensitivity of the test, values close to one indicate high sensitivity.
* = the parameter is significantly different from 0 ($P < 0.05$), ns = not significantly different ($P > 0.05$). Significance is only indicated where relevant.

From the estimated parameters a plot of the median waiting-time can be constructed, see Figure 2.

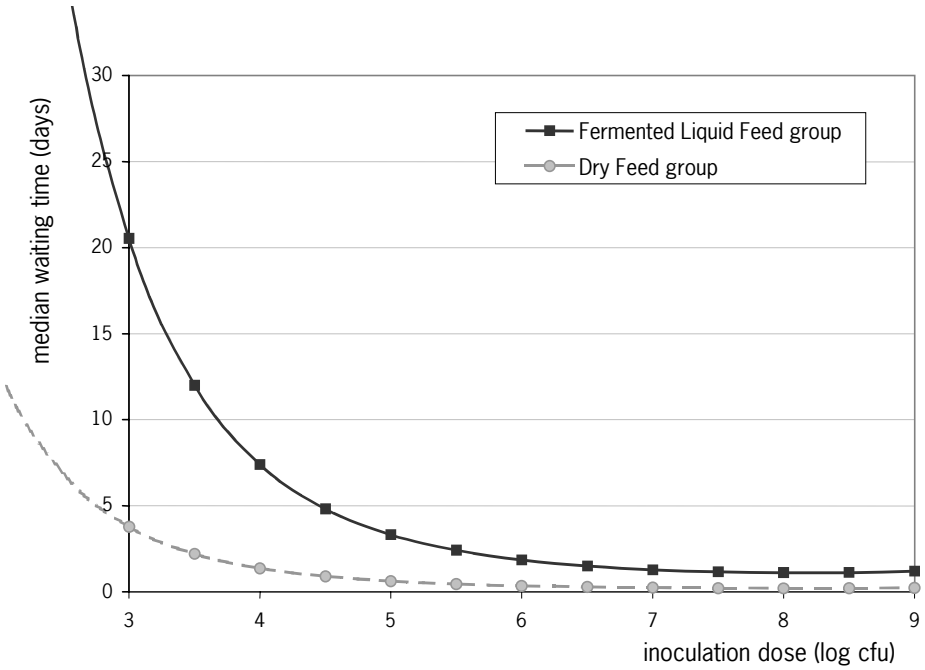

Figure 2. Median waiting time for *Campylobacter* shedding after inoculation with several doses based on pooled data of three experiments

Table 4 shows the comparison of the median waiting time with the outcome of other experiments with Salmonella (Heres et al. 2003a).

Table 4. Comparison of effect parameters in several experiments

	Estimated difference between proportion of infected chickens from a result table (in log cfu)	Measured difference in inoculation dose for a similar median waiting-time p.i. (in log cfu) - result of the statistical analyses	RR of control versus treatment ($e^{delta\ a}$) - result of the statistical analyses
FLF and *Salmonella* (Heres et al. 2003a)	3 to 4	1.7	3
FLF and *Campylobacter* (Heres et al. 2003b)	1 to 2	2	9

Discussion

For the infection experiments with individually housed chickens that were inoculated with different doses and where binary data were available for each individual chicken, we experienced that the Bayesian analysis employing Markov Chain Monte Carlo (MCMC) was quite useful for the statistical analyses. The Cox' proportional hazard reflected the typical features of the data, i. e. dependency, waiting-time structure and censoring. The outcome of the analyses is a measure of difference in susceptibility between the feed groups.

In Table 4 the different measures of protection are compared. The comparison of the protecting effect of fermented feed calculated with the survival analyses indicates that the effect of FLF is larger against *Campylobacter* than against *Salmonella*, whereas the differences between proportions of infected chickens were interpreted as a bigger effect against *Salmonella*.

The following must be taken into account for the discussion of the difference in interpretation between the comparison of proportion of infected chickens as shown in the result table (Table 3) and the difference of inoculation dose for a similar waiting-time based on the statistical analyses.

The comparison of proportion of infected chickens was mainly based on differences at one time point after inoculation, and especially the data of the first days. The statistical model takes all data into account and therefore seems superior. In retrospect the interpretation of differences in proportion of infected chickens was biased by overweighing the observed protective effect of fermented feed against *Salmonella* during the first three days. The model supposes that the number of chickens that shed *Salmonella* increases throughout the experiment. For *Campylobacter* however it is more that the number of infected chickens increases during the first 5 to 6 days and is stable thereafter. The data set was too limited to take this into account, for example by introduction of heterogeneity. This does not withstand that also for *Campylobacter* the analysis is the one that takes most characteristics of the data into account. For *Salmonella* the survival model may be more appropriate, because the number of shedding chickens rose throughout the experiment. Because the infection characteristics appear to be different for *Salmonella* and *Campylobacter* the comparison of both analyses must be done with caution.

An advantage of the MCMC method is its flexibility in conjunction with use of the Gibbs sampler, an instance of MCMC, as implemented in the BUGS programme (Spiegelhalter et al. 2003). Extensions of the model, e.g. inclusion of a sensitivity parameter, are easily included, without the need for a modification with extra safeguards of the fitting algorithm. Derived parameters, such as median waiting-time, are easily obtained. These are technical advantages of course. In the analysis we employed fairly non-informative priors. In subsequent research it is feasible to include prior knowledge through informative priors. This is an elegant feature of Bayesian statistics. Although not impossible, inclusion of prior information in a frequentist ML would be much harder to realize.

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