Quantitative Risk Assessment of *Campylobacter* in broiler chickens - assessing interventions to reduce the level of contamination at the end of the rearing period

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1. INTRODUCTION

Campylobacter is a major cause of foodborne disease worldwide (Havelaar et al., 2015). The pathogen is believed to be responsible for about nine million cases of human campylobacteriosis per year in countries of the European Union (EU), with an estimated cost to the EU economy of approximately EUR 2.4 billion per year (EFSA, 2015). Chicken meat is a well-known source of *Campylobacter*; in 2010 the European Food Safety Authority (EFSA) estimated that between 20% and 30% of the total cases of campylobacteriosis across the EU can be attributed to the handling, preparation and consumption of broiler meat while 50% to 80% may be attributed to the chicken reservoir as a whole (EFSA, 2010). Following a request from the European Commission, the Panel on Biological Hazards in 2011 issued a scientific opinion on *Campylobacter* in broiler meat production: the control options and performance objectives and/or targets at different stages of the broiler meat chain. The major conclusions were: (i) there is a linear relationship between prevalence of *Campylobacter* in broiler flocks and public health risk and (ii) reducing the numbers of *Campylobacter* in the intestines of chickens at slaughter by 3 log CFU/g units would reduce the public health risk by at least 90% (EFSA, 2011). The opinion concluded that controlling *Campylobacter* in primary broiler production would result in greater public health benefits than interventions at later stages in the food chain. Although the linearity of the relationship should be considered a simplification and interpreted cautiously, a recent review supports the hypothesis that mitigation strategies aimed at reducing the level of contamination of the birds entering the slaughterhouse would result in significant reduction of the risk for human health (Meunier, Guyard-Nicodème, Dory, & Chemaly, 2016). Increased understanding of the dynamics of within flock infection and of the likely impact of interventions on the level of contamination is therefore a public health priority.

Following these considerations, the aim of this study was to quantify the effect of farm-level mitigation strategies on the level of contamination of broiler flocks at depopulation. The proportion of Highly Contaminated Flocks (*%HCFs*) sent to slaughter was used as the unit of comparison. The threshold used to define a flock as 'Highly' contaminated was previously formulated by Georgiev at al. (Georgiev,

Beauvais, & Guitian, 2016) and used in their epidemiological study aimed at exploring factors associated with the risk of a broiler flock being highly colonized at slaughter in the UK. Three categories can be distinguished amongst the strategies that were explored: (i) management practices aimed at reducing chicken's exposure to the pathogen (enhancement of biosecurity, avoidance of partial depopulation, early final depopulation), (ii) interventions aimed at increasing the resistance of broiler chickens to colonization (e.g. through vaccination or use of feed additives) and (iii) mitigation strategies aimed at reducing the pathogen's load in the caecal contents of infected birds (bacteriophage therapy and bacteriocins). Factors that reflect the level of biosecurity (e.g. adopting rodent control around the broiler house, changing of footwear and clothes before entering the houses or improvement of the hygiene barriers), adoption of the thinning practice and the slaughter age, have been frequently identified as risk factors for *Campylobacter* colonization in broilers at slaughter (Allain et al., 2014; Bouwknegt et al., 2004; Georgiev et al., 2016; Hansson, Engvall, Vagsholm, & Nyman, 2010; Torralbo et al., 2014). Not surprisingly, control strategies exerting effects on those factors are placed in first position in the hierarchy of control methods reported by EFSA (EFSA, 2011). However, it should be noted that while factors like changing footwear or improving hygiene barriers are easier and relatively cheap to handle, avoiding thinning and earlier depopulation need rigorous cost-benefit analysis.

On the other hand, although results of experiments assessing the efficiency of mitigation strategies aimed at increasing resistance to colonization or reducing the level of the pathogen in caeca are encouraging (Meunier et al., 2016; Robyn, Rasschaert, Pasmans, & Heyndrickx, 2015), further studies to obtain more reproducible results are needed before effective applications of those measures on large scale.

As suggested by Robyn et al., lowering or delaying *Campylobacter* colonization in broiler flocks is likely to be more effective by combining measures directed to prevent the introduction of *Campylobacter* into the flock with measures aimed at lowering *Campylobacter* survival in infected broilers (Robyn et al., 2015).

In our model, the assessment was made by developing a baseline probabilistic model aimed at capturing the dynamics of the within flock transmission of *Campylobacter* in a typical broiler chicken flock and comparing the proportion of highly contaminated flocks obtained under baseline conditions with that obtained when different strategies were implemented. The study includes the findings of an epidemiological study conducted to support the activities of the UK's Food Standards Agency (FSA) and the UK Joint Working Group on *Campylobacter* that generated estimates of the strength of association between management conditions and likelihood of flock colonization at high levels (Georgiev et al., 2016)

2. MATERIALS AND METHODS

2.1. The baseline model. The baseline model, outlined in Figure 1, was aimed to estimate the proportion of flocks with average contamination level higher than 5.09 log/CFU g as a function of (i) the within flock prevalence (*WFP*) and (ii) the individual level of contamination (log CFU/g) in colonized birds. The baseline model was implemented with the available information and/or data included in studies related to broiler chicken raised in intensive systems in the UK (Georgiev et al., 2016; Goddard, Arnold, Allen, & Snary, 2014). The assessment of the mitigation strategies affecting the pathogen's load in the caecal contents of infected birds was made by adopting the overall effects of the interventions already summarized by EFSA (EFSA, 2011).

One of the main factors driving the model outcome, the *WFP,* can be expressed as the ratio between the number of birds colonized with *Campylobacter* over the total number of birds in the flock. This value is calculated at the day of final depopulation or clearance (*dpday*) and assumed to be dependent on two main factors:

1. The age or day of the cycle at which the flock became colonized
2. The spread of *Campylobacter* within the flock following colonization measured as the rate at which non-colonized birds become colonized

In our model, the first day of colonization defines the moment at which the spread starts, which is in turn dependent on a number of biological variables such as the total number of birds in the flock (*Nb*) and the number of infected birds at t_0 (It_0).

2.1.1. *The age at which the flock became infected.* The dynamics describing the broiler becoming colonized by *Campylobacter* and the time at which this occurs in a typical broiler flock are largely unknown. Longitudinal studies of broiler flocks raised under commercial conditions, have reported that *Campylobacter* is rarely detected before 10 to 14 days after the beginning of the production cycle (Bull et al., 2006; Evans & Sayers, 2000; Jacobs-Reitsma, van de Giessen, Bolder, & Mulder, 1995) and for modelling purposes, the first day at which the flock become colonized has been proposed to be adequately described as a uniform random variable between fourteen days and the day of depopulation (FAO/WHO, 2009; Hartnett, Kelly, Newell, Wooldridge, & Gettinby, 2001). While the assumption of the minimum age of flock infection is biologically plausible (i.e. presence of passive immunity) and supported by empirical data, assuming that infection is equally likely to occur on each day of the cycle after day 10 is in conflict with field evidence. Applying a Bayesian model to several longitudinal datasets on *Campylobacter* infection in UK broiler flocks, Goddard et al., estimated that the time at which a flock becomes infected with *Campylobacter* ranges between 10 and 45 days, with a most likely value around 30–35 days (Goddard et al., 2014); thus, we assume the first day of colonization ($Cday^+$) can be described as:

$$Cday^+ = Pert(Min, Max, Most\ likely) \quad \text{(Eq.1)}$$

Where Min=10, Max=45 and Most likely is a Discrete (30,31,32,33,34,35). Ten thousand iterations were run and the cumulative distribution obtained for $Cday^+$ used to estimate the daily probability of a flock becoming infected. Therefore, the chances that each day has to be $Cday^+$ were finally modelled as:

$$Cday^+ = Discrete\left(10, .., dpday; p_{10}^+, \dots, p_{dpday}^+\right) \quad \text{(Eq.2)}$$

Where *dpday* is the day of final depopulation and $p^+_{10} \dots p^+_{dpday}$ are the estimated probabilities according to (Eq.1).

2.1.2. *Spread of infection.* The spread of *Campylobacter* within the flock following its colonization on *Cday*$^{+}$ was assumed to exhibit logistic growth. The results of two experiments (Van Gerwe et al., 2005) were fitted to a logistic growth curve:

$$Ib_t = \frac{K\ Nb\ Ib_0}{Ib_0 + (kNb - Ib_0)e^{-rate*t}} \quad \text{(Eq.3)}$$

Where Ib_t is the number of colonized birds at time *t*, *Nb* is the flock size, *K* the carrying capacity of the environment (assumed equal to 1) and *rate* is the coefficient representing the growth rate of colonized birds in the total population.

In both the experiments, 400 broiler chicks were housed on fresh litter in a density of 20 chicks/m^2 and 4 chicks per group were orally challenged at the age of 2 days. The colonisation of chicks was determined at ten time points (i.e. day 4, 5, 7, 9, 12, 14, 28, 40 and 42) by collecting faecal samples from 50 random birds. When all the samples were found to be *Campylobacter* positive, the sample size was reduced to 10 chicks per group in both the experiments (Van Gerwe et al., 2005).

The parameterization of a logistic function was already used in a previous work (Katsma, De Koeijer, Jacobs-Reitsma, Mangen, & Wagenaar, 2007) where *rate* was estimated from the results of the original work (reported as number of positives observed in samples of size 50 and 10 birds) extrapolating the actual number of infected birds in the whole population (N=400) at each data point. Using the original dataset, we used the hypergeometric process to include the uncertainty surrounding the number of infected birds detected in each sampling time given the sample size. In fact, given that at different sampling time, samples of size n_i were collected from a finite population *M,* we parameterized the total number of infected $D_i(\theta)$ in the population at each time point *i*, given that s_i positive samples were observed. Assuming the uninformative prior for the parameter $\pi(\theta)=1$, the Likelihood of observing s_i infected for a given value of θ was estimated with the hypergeometric probability mass function:

$$L(s_i|n,\theta,M) = \frac{\binom{\theta}{s_i}\binom{M-\theta}{n-s_i}}{\binom{M}{n}} \quad \text{(Eq.4)}$$

Therefore, for each sampling time, the posterior distribution describing the actual state of knowledge about θ was estimated as:

$$f(\theta|x)_i \propto \pi(\theta) * L(s_i|n, \theta, M) \quad \text{(Eq.5)}$$

Indicating that the posterior distribution describing the expected number of colonized birds in the population at each i^{th} sampling point (x) is proportional to the prior believe about the parameter (π) and the likelihood function for a hypergeometric process expressing the calculated probability of observing s_i positive birds given n, M, and a given value of θ.

The distribution describing the number of colonized birds allowed the simulation of alternative outcomes for each i^{th} sampling point: ten thousand simulated datasets were fitted to the logistic growth function (Eq.3) and as many values for *rate* were obtained. To parameterize the distribution describing the uncertainty in *rate* from the values obtained, the maximum likelihood estimation (MLE) method for a Gamma distribution was used (Vose, 2008). Assuming that a given set of data can be described by a certain distribution (e.g. Gamma), the method of maximum likelihood provides an estimation of the distribution's parameter(s) so that the joint probability of the observed data under the resulting distribution is maximized:

$$\log L(X|\alpha) = \sum \log(f(xi, \alpha)) \quad \text{(Eq.6)}$$

Where α represents the parameter(s) of the distribution of the likelihood function (α and β of the Gamma distribution) and $\log L(X|\alpha) = \sum \log(f(xi,\alpha))$ is the likelihood of observing the n observations recorded given α. The gamma distribution was chosen because data are continuous and its parameters α (shape) and β (scale) allow great flexibility making possible for the distribution to assume a range of different shapes.

2.1.3. *Within flock prevalence estimation.* In each simulated scenario, the *WFP* was defined as the predicted proportion of infected birds on *dpday*. The probability distribution describing the *WFP* was obtained through the simulation of 500,000 production cycles in which $Cday^{+}$ was randomly sampled

according to Equation 1, and the spread of the infection modelled by fitting a logistic growth model in which the coefficient *rate* was sampled from its uncertainty distribution.

2.1.4. *Infected birds in infected flock at slaughter.* The actual number of infected birds in the flock *N(Ib)* was estimated after each iteration as:

$$N(Ib)_i = Nb * WFP_i \quad \text{(Eq.7)}$$

Where *Nb* is the number of birds in the flock and WFP_i is the estimated within flock prevalence in the flock after iteration i^{th}.

2.1.5. *Level of contamination of the flock.* The level of contamination of the flock is generally estimated by bacteriological count of a number of pooled caeca (N_c) randomly sampled at the slaughterhouse, therefore, the final result can be assumed to be a function of: (i) the number of contaminated caeca sampled and (ii) the level of contamination in a positive sample.

2.1.6. *Number of contaminated caeca samples.* The Hypergeometric process was used to estimate number of contaminated caecal sampled (N_c^+) as a function of *Nb*, N_c and $N(Ib)_i$:

$$N_c^+ = Hypergeometric\ (N_b; N(Ib)_i; N_c) \quad \text{(Eq.8)}$$

2.1.7. *Level of contamination in caeca.* The ability of *Campylobacter* in reaching high level in caecal contents after infection has been widely reported (Nauta, Jacobs-Reitsma, Evers, Van Pelt, & Havelaar, 2005; Shanker, Lee, & Sorrell, 1990; Uyttendaele et al., 2006). The Intestinal carriage of *Campylobacter* in contaminated chicken carcasses entering the slaughterhouse (C_c) was estimated from a previous study (Rosenquist, Sommer, Nielsen, & Christensen, 2006) and assumed to be adequately described by the normal distribution:

$$C_c = Normal(\mu_c; \sigma_c) \quad \text{(Eq.9)}$$

With parameters μ_c and σ_c equal to 7.63 and 1.02 log CFU/g respectively. The final level of contamination of the flock (*Fl*) was inferred from the estimated level of contamination of a standard pooled sample of 10 caeca samples/batch:

172 $$Fl = \frac{Normal((\mu_c * N_c^+); \left(\sqrt{N_c^+} * \sigma_c\right))}{N_c} \quad \text{(Eq.10)}$$

173 Where the numerator represents the central limit theorem applied on the positive caeca samples taken
174 (i.e. it is assumed that the level of contamination in each positive sample included in the pool can be
175 described by the same distribution), and the denominator the total number of caeca samples. A test
176 sensitivity close to 100% is assumed. The practical sample size of 10 caeca sample was selected for N_c
177 (FSAI, 2011). The impact of N_c on *Fl* was explored by scenario analysis in which the arbitrary values for N_c
178 [50; 100; 200; 500; 1000; 5000; 1000] were selected.

179 **2.2. Baseline settings.** In the baseline model, 500,000 infected flocks were simulated. It was
180 assumed that each flock was raised in a broiler house with 20,000 birds (*Nb*), under a standard
181 biosecurity (B-), without partial depopulation (T-). The simulation was initiated assuming that the
182 infection was due to one initially colonized chicken -shedder- (Ib_0=1*)* and according to the industry
183 dataset (Georgiev et al., 2016), the thirty-eighth day of the cycle was selected as the most likely day of
184 clearance (*dpday*) in flocks that were not partially depopulated. The inputs of the baseline model are
185 resumed in Table 1.

186 *Table 1 overview of the input parameters included in the baseline model. Assumed distribution and data source are reported.*

Input	Distribution/Function	Description	Source
$Cday^+$	Pert (10, 45, Most likely) With: Most likely = Discrete (30,31,32,33,34,35)	First day of infection	(Goddard et al., 2014)
dpday	38	Day of depopulation	(Georgiev et al., 2016)
rate	Gamma (652.2;0.0010)	Coefficient of the logistic curve: $Ib_t = \frac{K\ Nb\ Ib_0}{Ib_0+(kNb-Ib_0)e^{-rate*t}}$	Fitted to experimental data (Van Gerwe et al., 2005)
Nb	20,000	Number of birds in baseline flock	Baseline constant
It_0	1	Number of infected at t_0	Baseline constant
$N(Ib)_i$	$Nb * WFP_i$	Number of infected in each i^{th} iteration	Model outcome
N_c	10	Number of caeca sample in the pool	Baseline constant
N_c^+	Hypergeometric (Nb; $N(Ib)_i$; N_c)	Number of infected caeca sample in the pool	Model outcome
C_c	Normal (7.63, 1.02)	Level of contamination in caeca (log CFU/g)	(Rosenquist et al., 2006)

Fl	$\frac{Normal((7.63*N_c^+);\left(\sqrt{N_c^+}*1.02\right))}{N_c}$	Average level of flock contamination (log CFU/g)	Model outcome

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188 **2.3. Risk outputs.** At the end of the simulation, the cumulative probability distribution obtained for
189 *Fl* was used to estimate the expected proportion of highly contaminated flocks at slaughter (*%HCFs*).
190 Once the baseline output was obtained, different management conditions and mitigation strategies
191 were tested and results compared to the baseline scenario. Moreover, in order to assess the relative
192 effects on the output of the inputs described by probability distributions ($Cday^+$; C_c; *rate*); a sensitivity
193 analysis was performed and tornado charts used to show the inputs ranked by effects on the output
194 mean.

195 **2.4. Measures to prevent chicken's exposure.**

196 **2.4.1.** *Enhanced biosecurity.* The relationship between enhancement of farm biosecurity and risk of
197 flock colonization has been established among others, in a recent epidemiological study (Georgiev et al.,
198 2016) where the adjusted Relative Risk (RRa) expressing the ratio of the probability of colonization in
199 farms with standard biosecurity vs. farms with enhanced biosecurity was obtained. Results of that study
200 indicate that batches raised under standard biosecurity are significantly more likely to be colonised at
201 high level than batches raised under enhanced biosecurity (RRa= 1.30 (95% CI: 1.05 – 1.48). Since the
202 baseline model assumed a standard level of biosecurity (B^-), the effect of enhanced biosecurity on the
203 proportion of highly contaminated flocks at slaughter was obtained using the RRa as multiplicative
204 coefficient as follows:

205 $P(B+T-) = P(B-T-)*1/RRa_{(B-)}$ (Eq.11)

206 Where, (B-T-) is the proportion of highly contaminated flocks obtained from in the baseline model. In
207 this case, the scenario (B+T-) estimates the proportion of highly contaminated flocks at slaughter if all
208 the infected flocks were grown under enhanced biosecurity management.

209 **2.4.2.** *Thinning.* Similarly to biosecurity, the estimated RRa for the factor of thinning (T+) resulted 1.55
210 (CI 1.18-1.87) for the flocks grown under enhanced biosecurity management. In the baseline model the

partial depopulation was not practiced, therefore, the effect of thinning on the proportion of highly contaminated flocks was estimated through the scenario (B-T+) in which 100% of the flocks are thinned before the end of the production cycle:

$$P(B\text{-}T+) = P(B\text{-}T-)*RRa_{(T+)} \quad \text{(Eq.12)}$$

An additional scenario (B+T+) in which the flocks are all assumed to be partially depopulated and raised under enhanced biosecurity measures was also assessed.

$$P(B+T+) = P(B\text{-}T-)* RRa_{(T+)}*1/RRa_{(B-)} \quad \text{(Eq.13)}$$

2.5. Measures to increase resistance to colonization. The interventions aimed at increasing resistance to *Campylobacter* colonization include the use of additives such as organic acids and phytocompounds in drinking water or feed, vaccination, and selective breeding (EFSA, 2011). Those measures are expected to reduce or even prevent colonization. In either case the result would be a reduction in the number of birds being colonized and thus, the *WFP*. It should be noted that despite significant progress, vaccines are still in the development phase and that the effects reported for other options are highly variable and characterized by variable or counterdicotry results and/or limited *in vivo* experiments (Hermans et al., 2011; Meunier et al., 2016). Assuming that those strategies would exert their effects on the spread of infection (*rate*, Eq. 3), we assessed the reduction of *WFP* as a function of the expected increase of the resistance to colonization. To this end, the increase of resistance was represented as a decrease in *rate* and results of *%HCFs* in scenarios with the parameter arbitrary decreased by 1%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70% were compared.

2.6. Measures to reduce the microbial load in colonized animals. The Interventions aimed at reducing the bacterial load in infected birds have been recognized as important on-farm mitigation strategies to reduce the average microbial load in contaminated flocks at slaughter (EFSA, 2011) and the available options such as the use of bacteriophage and bacteriocins have been very recently reviewed (EFSA, 2011; Meunier et al., 2016; Robyn et al., 2015). The efficacy of those interventions depends on a number of biological and technical factors and their effect is still difficult to estimate quantitatively

(Hermans et al., 2011). For this reason, a generic modelling approach to evaluate the reduction in *%HCFs* at slaughter due to a reduction in the pathogen load in intestines was performed to assess the potential benefit of interventions with this general aim. Adopting the values reported in the EFSA scientific opinion (EFSA, 2011), the assumed effects on *Campylobacter* reduction in intestine of colonized birds were fixed to 3 log CFU/g and Uniform(5,1;5,9) log CFU/g for a generic treatment with bacteriophages and bacteriocins respectively. In the model, both mitigation strategies affecting the level of contamination in infected birds are assumed to act on individual μ_c (Eq.9). Considering that the reductions in caecal load described above are only rough approximations of the expected effects and that the effect of such mitigation strategies on *%HCFs* cannot be directly inferred by coefficients, simulations were used to explore the general relation describing the changes in *%HCFs* as a function of: (i) the expected reduction on the caecal load and (ii) the within flock prevalence. To this end, different values for the expected reduction effect (from 0.25 to 4 log UFC/g by increment of 0.25 log/CFU/g) were tested against different levels of *WFP* (from 0.1 to 1 by 0.025) and results of generated scenarios assessed. A similar approach was used for the day of final depopulation; as *dpday* is one of the few inputs of the model directly influenced by the management; changes in *%HCFs* as a function of a change in this parameter was assessed simulating 10 different scenarios (*dpday* ± 5days).

2.7. Uncertainty in the baseline scenario. The effects of the interventions under investigation on *%HCFs* were estimated by comparing the outputs of the different scenarios obtained by means of Monte Carlo Simulations with that of the baseline. The effects were estimated using a standard broiler flock as baseline; therefore, certain flock characteristics were assumed and although the production process of broiler chickens is highly standardized, in reality, some inputs such as *Nb, d_rate* or *dpday* might be different amongst the farms. The same applies to the initial number of infected at $Cday^{+}$ which is intuitively strictly dependent on the source of *Campylobacter* infection and for which the effect is typically unknown. Those inputs are expected to have an impact on the *WFP* and consequently on *Fl* and *%HCFs* (Figure 1), therefore, to quantify those effects, the baseline values were replaced by distributions (Table 2) describing the variability and the uncertainty surrounding the parameters. The output of the

model obtained with those inputs was used to perform a sensitivity analysis and tornado charts were used to represent $Cday^{+}$, Nb, It_0, C_c, $dpday$ and $rate$ ranked by effect on the output mean. The distributions describing Nb and was obtained assuming a conservative discrepancy of ±100% from the baseline information while the effect of the uncertainty surrounding the initial number of shedders was tested assuming that It_0 may range from 0.05% (It_0=1) to 5% (It_0=1000) of the total population. The day of final depopulation depends on several biological, economical and practical factors; industry data were used to estimate the parameters (Minimum; Most Likely; Maximum) of the Pert distribution describing the uncertainty in *dpday*.

Table 2 distributions used to evaluate the impact of the input on the model output.

Input	Unit	Distribution	Assumption
Nb	Unit	Uniform(5,000;40,000)[1]	±100% discrepancy from the baseline
It_0	%	Uniform (0.5;5)	+100% discrepancy from the baseline
dpday	Unit	Pert (36;38;50)	Industry data (Georgiev et al., 2016)

[1]The minimum values of 5000 and 1% were maintained for the uncertainty distribution representing *Nb* and *d_rate respectively*.

Simulation. The risk analysis software @Risk (version 7.0.1 for Excel, Palisade Corporation, Newfield, NY) was used for the simulations and sensitivity analysis. Statistical software R 3.3.0 was used for the graphs.

3. RESULTS

3.1. Baseline model

Following the flowchart reported in Figure 1, the following results were obtained for the steps driving to the proportion of highly contaminated flocks in the baseline model.

3.1.1. *Age at which the flock became infected.* The cumulative probability distribution representing the chances of a given day being the day of infection is presented in Figure 2. The distribution indicates that there is a probability of 35.67% that the day of infection falls in the range 10-28 days, 73.06% in the range 10-35 days and 98.35% in the range 10-42 days.

3.1.2. *Spread of infection.* Following the estimation of the parameters obtained by the MLE (Eq.6), the Gamma distribution describing the *rate* resulted:

$$rate = Gamma(652.2; 0.0010) \quad \text{(Eq.14)}$$

The distribution shows a mean of 0.698 with a standard deviation of 0.027. The effect of the uncertainty surrounding the parameter when the logistic growth model was adapted to the baseline scenario, (Nb=20,000 chicken broilers with one initial infected at t_0) is shown in Figure 3. Assuming $t_0 = 0$ for the purpose of illustrating the effect of the variability and the uncertainty, it takes from two to three weeks from the day of infection before the *WFP* reaches the 100%.

3.1.3. Within flock prevalence.

Over 100,000 simulated flocks, the *WFP* at slaughter resulted equal to 46.35% on average. The cumulative distribution together with the probability density is reported in Figure 4. The *WFP* was below 50% in 72.4% of simulated scenarios and close to 90% at 90th percentile.

3.1.4. *Level of contamination.* The cumulative distribution describing *Fl* (Eq.10) for the baseline model is reported (Figure 5). In the baseline model, the average value recovered for *Fl* was 1.83 log CFU/g, with a standard deviation of 2.7 log CFU/g. The value at 95th percentile was 7.6 log CFU/g with 18.8% of infected flocks showing a contamination greater to 5.09 log CFU/g. The result of the sensitivity analysis outlined as tornado chart with the inputs ranked by effect on the output mean is reported in Figure 6. Considering that *Fl* is calculated from the estimated level of contamination of a pooled sample (Eq.10), this value is directly dependent on the number of infected birds in the flock (Eq.8-9). In fact, the tornado chart clearly shows that the $Cday^+$ (which determines *WFP*) is the input with the greater influence on the output. On the other hand, the parameters *rate* and the distribution describing C_c shown a limited impact on *IF*, in fact, the average of *Fl* ranged from 1.52 to 2.11 log CFU/g as a function of *rate* and from 1.80 to 1.87 log CFU/g as a function of C_c. When different values for N_c were simulated, significant differences in the model's output were not observed with the *%HCFs* resulting 17.9% when 10000 caeca samples were used.

3.2. Effects of mitigation strategies

The estimated *%HCFs* for the scenarios in which enhanced biosecurity (B+T-), partial depopulation (B-T+) or both management options were enabled (B+T+), are reported in Table 3. The confidence limits associated to the RRa of the factors under investigation were used in Eq.11-13 so that the 'best' and the 'worst' scenarios reflecting the uncertainty surrounding the estimates were reported.

Table 3 resulting proportion of flocks included in the category '>5.09 log CFU/ml' at slaughter when the effect of management conditions affecting the introduction of pathogen and/or the spread of the infection (enhanced biosecurity, thinning) were simulated. Numbers in brackets represent the ±deviation from the baseline output in percentage.

		%HCFs*	
Scenario	**Output**	**BEST SCENARIO**	**WORST SCENARIO**
Baseline (B-T-)	18.8%	//	//
B+T-	14.4% (-23.4%)	12.7% (-32.44%)	17.9% (-4.78%)
B-T+	29.1% (+54.78%)	22.1% (+17.55%)	35.1% (+86.70%)
B+T+	22.4% (+19.14%)	15.0% (-20.21%)	33.4% (+77.65%)

*proportion over 500,000 simulated flocks

As expected, the application of biosecurity measures reduced the predicted *%HCFs*. Conversely, the thinning practice had a negative impact. Interestingly, when both, the biosecurity measures and the thinning practice were adopted, the combined effect of the factors was not conclusive, in fact, the uncertainty surrounding the effects led to a reduced and increased proportion of highly contaminated flocks when the best and the worst scenarios respectively were assessed. For each on-farm mitigation strategy aimed at reducing the microbial load in colonized animals, the distributions describing *Fl* (mean, 5^{th} and 95^{th} percentile) and *%HCFs* are reported in Table 4.

Table 4 results obtained for Fl and %HCF when the effect of interventions aimed to reduce the bacteria load in infected birds were simulated. Numbers in brackets represent the ±deviation from the baseline output in percentage.

	***Fl* (log CFU/g)**			***%HCFs****
	Output (mean)	**5^{th} p.ile**	**95^{th} p.ile**	**Output**
Baseline (B-T-)	1.83	0.00	8.65	18.8%
BACTERIOCINES	0.504	0.00	2.56	0% (-100%)
BACTERIOPHAGE	1.10	0.00	4.59	0.06% (-99.6%)

*proportion over 500,000 simulated flocks

The graph representing the changes in *WFP* as a function of different expected resistance (expressed as decreasing *rate*) against *Campylobacter* colonization are reported in Figure 7, while the respective effects on the distributions describing *Fl* (mean, 5th and 95th percentile) and *%HCFs* are reported in Table 5.

Table 5 results obtained for Fl and %HCFs when the effect of interventions aimed at enforcing the individual resistance to Campylobacter were tested. Numbers in brackets represent the ±deviation from the baseline output in percentage.

	***Fl* (log CFU/g)**			***%HCFs****
Decrease in rate (% Baseline)	**Output (mean)**	**5th p.ile**	**95th p.ile**	**Output**
Baseline	1.82	0.00	8.65	18.8%
-1%	1.78	0.00	7.57	18.21% (-3.08%)
-5%	1.61	0.00	7.47	15.34% (-18.34%)
-10%	1.38	0.00	7.26	12.55% (-33.20%)
-15%	1.16	0.00	6.85	9.80% (-47.82%)
-20%	0.94	0.00	6.15	7.22% (-61.58%)
-30%	0.54	0.00	3.83	2.92% (-84.43%)
-40%	0.24	0.00	1.59	0.56% (-97.03%)
-50%	0.08	0.00	0.75	0.01% (-99.92%)
-60%	0.02	0.00	0.00	0.00% (-100%)
-70%	0.01	0.00	0.00	0.00% (-100%)

*proportion over 500,000 simulated flocks

The graph representing the general relationship of the reduction in *%HCFs* as a function of the expected reduction effect on the caecal load (-log CFU/g) and *WFP* is reported in Figure 8 and that showing the expected reduction in *%HCFs* as a function of *dpday* (±1-5days) is presented in Figure 9.

As expected, *%HCFs* is greatly impacted by the transmission rate; a reduction of 10% in the rate of transmission led to a 50% decrease of the probability of highly contaminated flocks at slaughter with respect to the baseline.

Similarly, the general relationship explaining the reduction in *%HCFs* as a function of *WFP* and the reduction of the level of contamination in caeca, clearly indicated how more drastic effects are needed from mitigation strategies operating on the individual level of contamination in caeca if the *WFP* is high.

Anticipating the day of depopulation by 1 day would lead to a 15% reduction of the estimated baseline value for *%HCFs*, while the delay of one day, lead to an increase of about 17% with an overall proportion of *%HCFs* of ~ 22%.

Uncertainty in the baseline scenario. In order to evaluate the effect that the fixed inputs have on the model output, the baseline values were replaced by the distributions reported in Table 2 and a sensitivity analysis was performed (Fig. 10).

In this case, $Cday^{+}$ remained the input with the greatest effect on the output mean, the uncertainty and variability underling the newly introduced inputs are likely to have a non-negligible effect on *FI*.

4. DISCUSSION

In this work we implemented a stochastic model that can be used to quantify: (i) the effect of mitigation strategies for which the specific point in time during the cycle and magnitude of the effect are both known and (ii) the effects of factors for which the specific point in time when the effect takes place is unknown but the overall effect on the output at the end of the cycle is known.

When targeted mitigation strategies aimed at reducing the bacterial load were tested, results clearly indicated that under the potential effects assumed by the model, treatment with bacteriocins and bacteriophages are consistently effective in reducing the level of contamination at individual level and thus *%HCFs* at slaughter. However, great care should be taken in considering these estimations; as previously remarked, the effects of those mitigation strategies were estimated by experimental trials in controlled environment and might not have captured variability under field conditions. Nevertheless, research efforts on measures to combat the survival of *Campylobacter* in colonized broilers are still ongoing and seem to be promising (Gracia et al., 2016; Guyard-Nicodeme et al., 2016; Hammerl et al., 2014; Robyn et al., 2015), the simple approach proposed to quantify those effects might be easily applied as soon as new evidence will be available. Similarly, despite the encouraging results of the experimental studies conducted so far, vaccines and other immunization strategies aimed at enforcing

the resistance against colonization by neutralizing and eliminating the pathogen at mucosal level are not available yet (Meunier et al., 2016). Results reported in Table 5 and Figure 8 provide a general understanding about the magnitude of the impacts on *WFP* as a function of a generic treatment aimed at decrease within flock transmission.

On the other hand, the coefficients used to correct the baseline estimation as a function of the adoption of enhanced biosecurity measures or/and the practice of partial depopulation were obtained from an exhaustive epidemiological study conducted in UK in 2014; therefore, these estimates are likely to represent effects under field conditions. At this respect, it should be noted that the results recovered for the scenarios under investigation (B-T-; B+T-; B-T+ and B+T+) were obtained assuming that all the simulated flocks operated at the same conditions. However, if the actual proportions of flocks operating under different management practices in the population are known, those fractions might be used to weight the results and obtain an estimation of *%HCFs* in the whole population.

It can be reasonably assumed that the general effects related to enhanced biosecurity measures are exerted on the parameters governing the *WFP* (*Cday*$^{+}$; *rate*) rather than *Fl*; this is supported by some recent findings in which chickens kept in an experimental 'bio-secure cube' become infected several days later (or remained *Campylobacter*-negative) than those kept in standard environment (Battersby, Whyte, & Bolton, 2016). Furthermore, results of a systematic review on on-farm sources of *Campylobacter spp.* concluded that the factors increasing the risk of contamination of a new flock seem to be related to biosecurity aspects such as insufficient cleaning and disinfection, insufficient downtime, and the presence of an adjacent broiler flock (Agunos, Waddell, Léger, & Taboada, 2014). Recently, Sommer et al., conducted a cross country study (Sommer et al., 2016) to identify on-farm risk factors for the colonization of broiler flocks with *Campylobacter* and confirmed the on-farm factors associated with the level of biosecurity as significant. On this basis, the general relationship displayed in Figure 8, can be considered as graphical evidence in support of the benefits that could be obtained when mitigation strategies operating at different levels are applied simultaneously. In fact, if biosecurity measures, or

strategies aimed at reducing the *WFP* are in place, less drastic mitigation strategies operating on the level of contamination are required to get a significant effect on the occurrence of *%HCFs*.

The on-farm model, although relatively simple, provided an exhaustive understanding of the dynamics leading to the *WFP* and the *Fl* in infected flocks and the related biological factors involved (i.e. *Nb*, It_0, *rate*, and C_c). The data used by Goddard et al. to parameterize the Bayesian model were collected from epidemiological studies related to commercial broiler chickens in UK; therefore, we believe the distribution describing $Cday^+$ can be considered as an acceptable approximation to describe the first day of *Campylobacter* infection in the UK broiler chicken flock.

For the transmission of *Campylobacter* within the flock, the logistic growth model proposed by Katsma et al. was adopted (Katsma et al., 2007), the main difference is that the Bayesian method allowed us to describe the parameter *rate* as a distribution instead of a fixed value. This gave us the opportunity to formerly consider the uncertainty underlying this input and assess its influence on the outcome by means of sensitivity analysis.

The on-farm model was developed not only with the intent of being a flexible and easily reproducible tool for the assessment of the mitigation strategies at farm level, but also for the quantification of the impact that variations in the baseline characteristics of a broiler flock might have on the outputs generated. A number of baseline information (*Nb*, It_0 and *dpday*) were included in the model as initiative inputs (Eq.3, *WFP*) and the potential impact on the outcome as a function of a variation in those values should be taken into account. In fact, the sensitivity analysis reported in Figure 9, clearly showed how variations in those inputs might lead to significant consequences; as a practical example, if *dpday* is anticipated by two days or *Nb* decrease by 5000 units, the baseline proportion of *%HCFs* decreased by 28% and increased by 8,7% respectively (results not shown). With respect to this, particularly useful is the general trend reproducing the changes in *%HCFs* as a function of *dpday* outlined in Figure 9. As previously highlighted, *dpday* is only dependent on production management and the expected *%HCFs* sent to abattoirs should be an integral part of the economic rationale behind the choice of *dpday*. The flexibility of the model leads itself to be applied to different settings if the baseline key parameters and

role of other risk factors are known. In addition, it should be noted that the same model can easily adapted to be used by growers to estimate the likely level of contamination of the batches entering the growing cycle.

Main assumptions and limitations. As in any model aimed to describe the complexity of a real system, some assumptions and limitation are recognized. The first assumption is related to $Cday^{+}$ where the baseline model assumes that the transmission never starts before the tenth day of the cycle. The sensitivity analysis (Figure 6-9) highlighted the importance of this input, but the threshold assumed by the model finds its justification from epidemiological data and biological characteristics such as passive immunity (Lin, 2009; Newell & Fearnley, 2003). However, if new evidence and data become available, the model can be easily updated operating on Equation 1. Another assumption is that the simplified transmission model does not admit that infected birds can recover. Even tough cases of self-limitation of the infection have been occasionally reported (Glünder, Neumann, & Braune, 1992), considering the chicken broiler reared in intensive system and the length of the production cycle (usually less than 40 days), It is generally accepted that once a bird is infected the infection persists until clearance.

An important limitation highlighted by the sensitivity analysis, concerned the effect of the uncertainty related to It_0. Our transmission model, was initiated assuming one initial infected bird but in reality, the initial number of shedders is likely to be strictly related to the source of contamination (i.e. if the source of contamination is the drinking water rather than faeces of wild animals, the number of infected birds at t_0 is likely to be very different). The identification of the on-farm risk factors for the introduction of *Campylobacter* has been assessed in several studies using structured questionnaires (Evans & Sayers, 2000; Gibbens, Pascoe, Evans, Davies, & Sayers, 2001; Hald, Wedderkopp, & Madsen, 2000; Refregier-Petton, Rose, Denis, & Salvat, 2001) but the relationship between source of contamination and number of infected birds at t_0 has never been formally investigated; this information can be easily included once available. Given the potential impact of this factor, further research focused on this relationship are strongly needed.

Conclusion.

In this work we explicitly accounted for all the main aspects involved in *Campylobacter* contamination at flock level and shown how expected effects of different mitigation strategies can be included in quantitative risk assessment models. The level of contamination of the flocks at the end of the rearing period is a well-known critical factor with recognized effects on human health; the results we provided highlighted how understanding the role and relationships of the individual inputs involved in the occurrence of highly contaminate flocks is crucial. The results reported, the identified relationships together with the structure of the model itself are practical instrument at the service of decision maker.

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Figure captions

Figure 1 Flowchart of the model implemented to assess the probability of an infected flock being classified as 'highly contaminated' at the end of the rearing period. The steps describe the baseline scenario in which simulated flocks are raised under a standard biosecurity regime and not thinned during the production cycle (B-T-). Additional scenarios, involving thinning (T+) and/or the application of biosecurity measures (B+) were assessed operating on the baseline estimation.

Figure 2 Cumulative probability describing the day of infection in positive flocks at slaughter. Infected flocks have 35.67%, 73.03%, and 98.35% probability of becoming infected by days 28, 35 and 42 respectively.

Figure 3 The effect of the uncertainty in the coefficient 'rate' on the horizontal spread. If the infection starts at day 0, the day at which the flock reaches a WFP of 95% ranges from day 15 to day 20 (dotted lines) because of the uncertainty surrounding the parameter.

Figure 4 Cumulative distribution and overlapped frequency of the baseline WFP at slaughter. The probability density is reported on the y-axis on the left and the cumulative distribution on the y axis on the right.

Figure 5 cumulative distribution of Fl; the reference line shows the contamination threshold after which flocks are considered 'highly contaminated' (i.e. 5.09 log CFU/g). Following simulation of 500,000 flocks, 18,8% of them fall above the threshold.

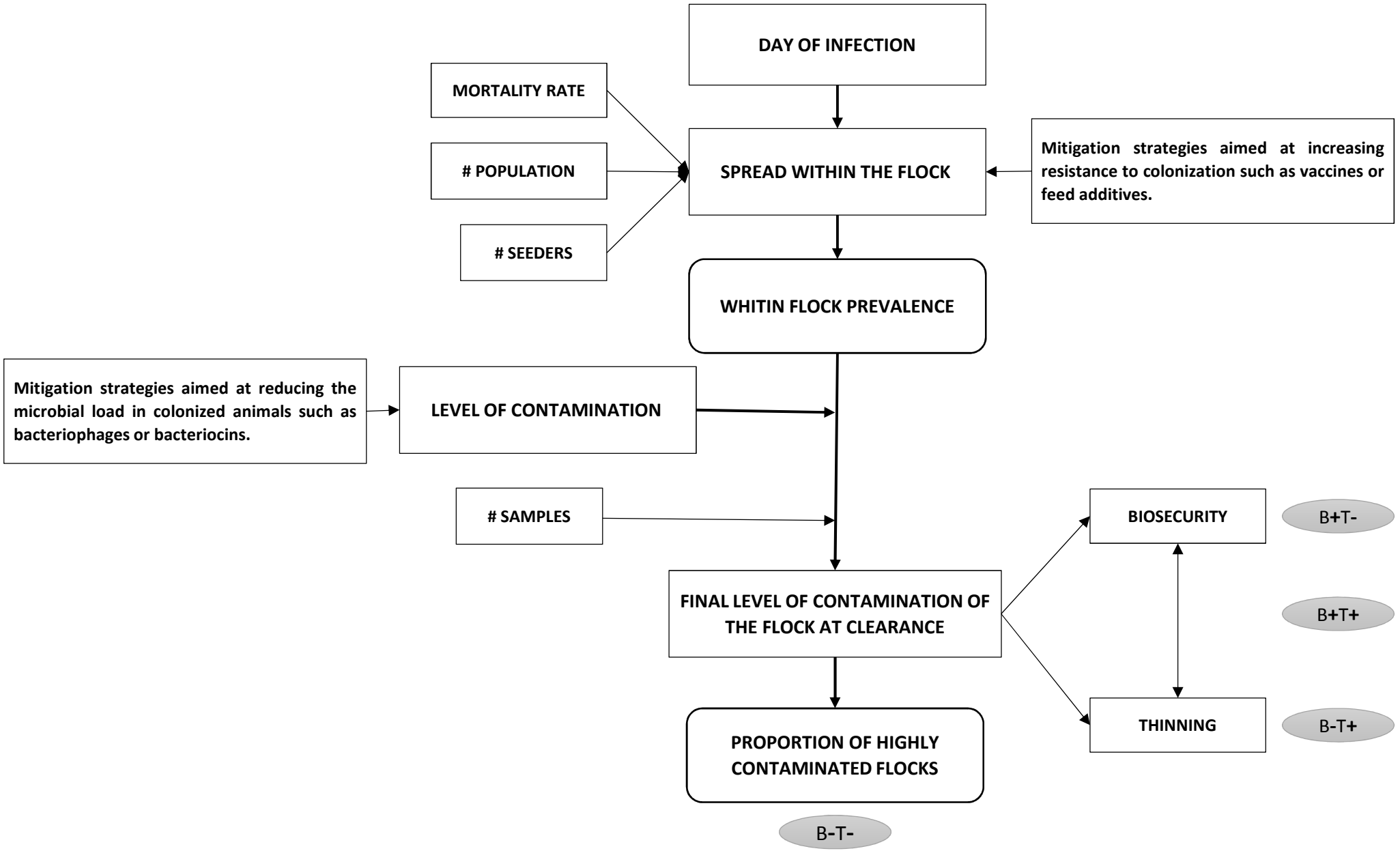
Figure 6 Tornado chart representing the model inputs ranked by effect on the output (Fl) mean. Each bar represent how much the respective input is able to displace the mean of Fl when all the others are fixed to theirs baseline value.

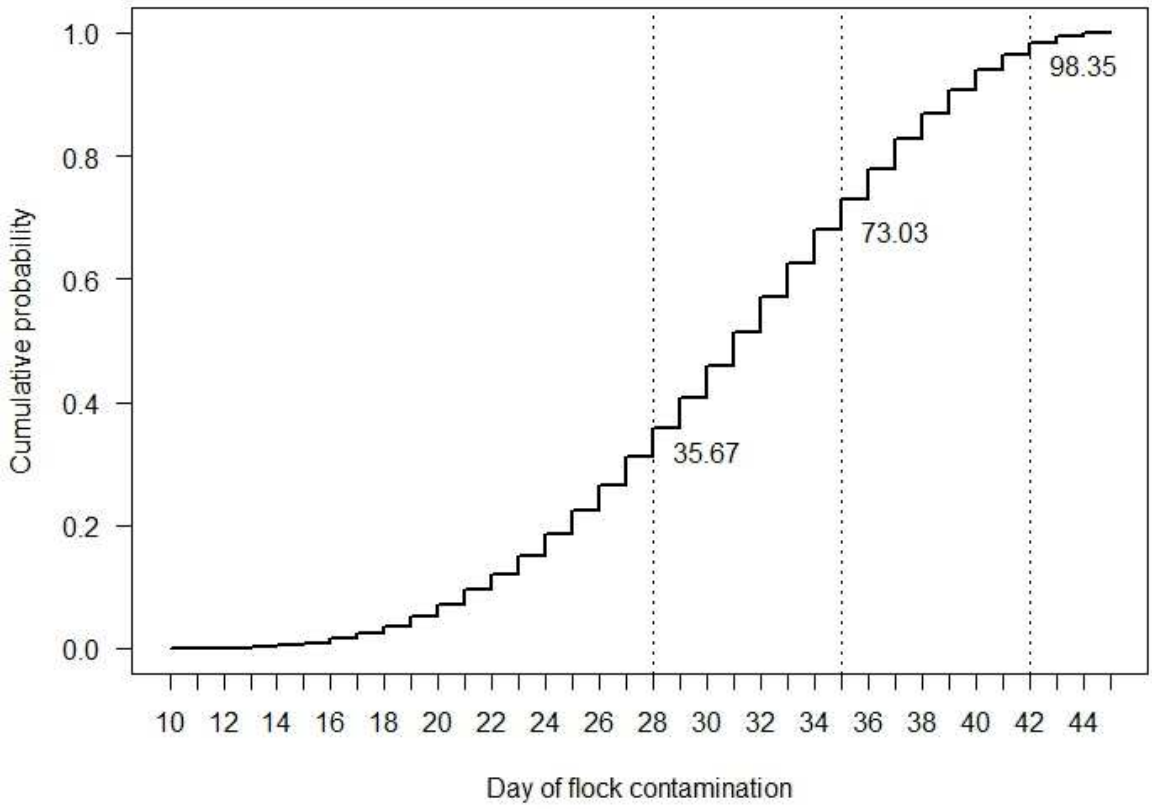
Figure 7 graphical reproduction of the change in WFP as a function of the within flock transmission due to mitigation strategies aimed at increasing the resistance to Campylobacter colonization. Ten cumulative distributions for WFP obtained simulating as many effects on the parameter 'rate' are reported.

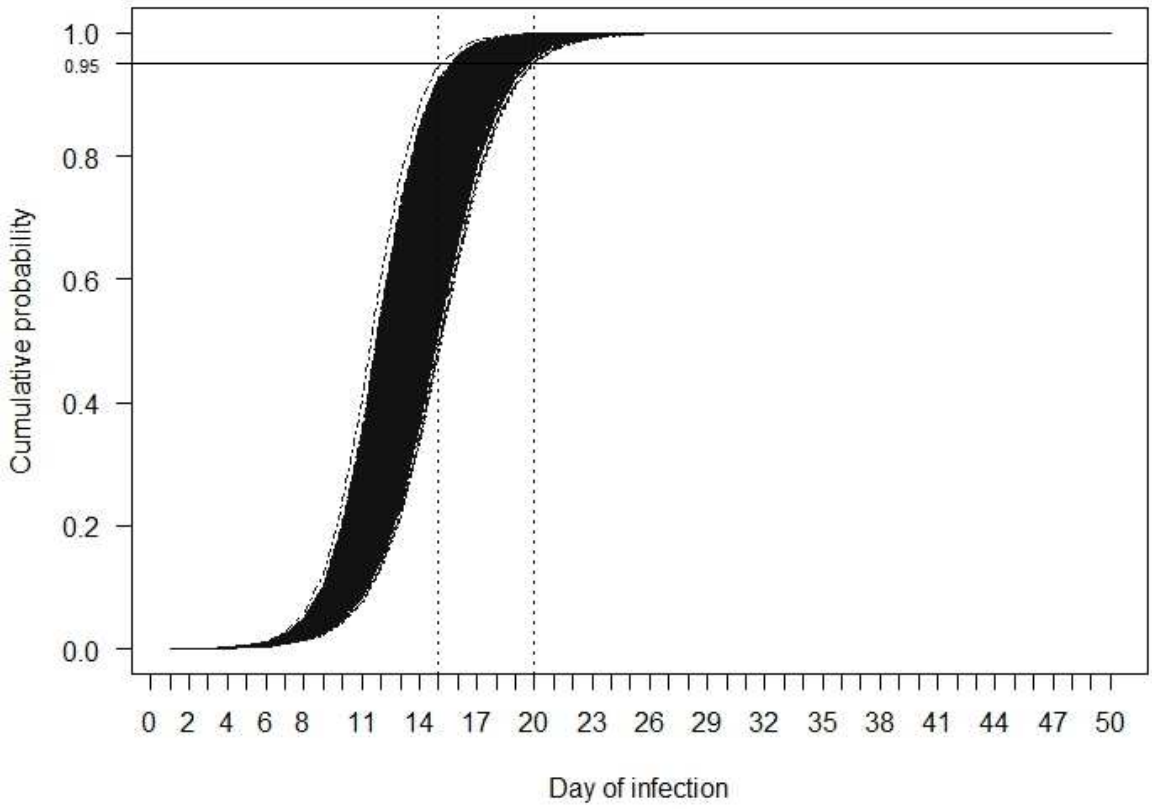
Figure 8 graphical reproduction of the change in the proportion of highly contaminated flock (-%HCFs) as a function of: (i) the expected reduction effect on the caecal load (-log CFU/g) and (ii) the within flock prevalence (WFP).

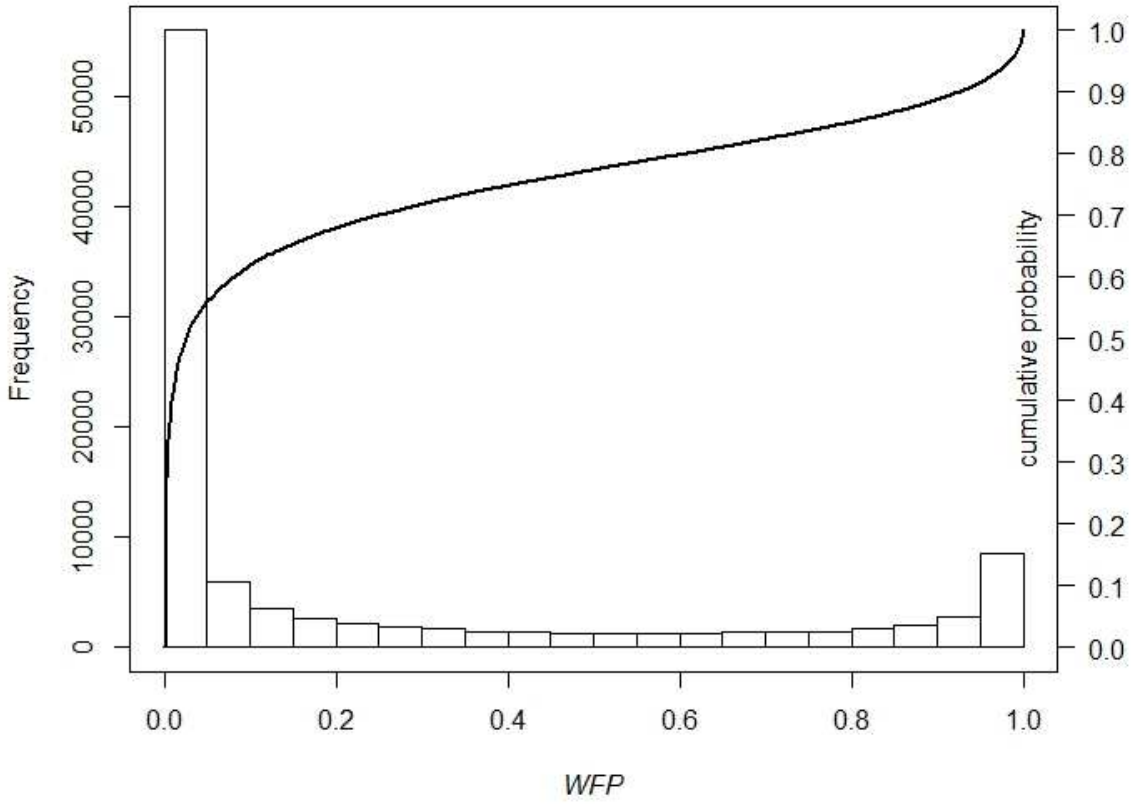
Figure 9 graphical reproduction of the change in %HCFs as a function of dpday.

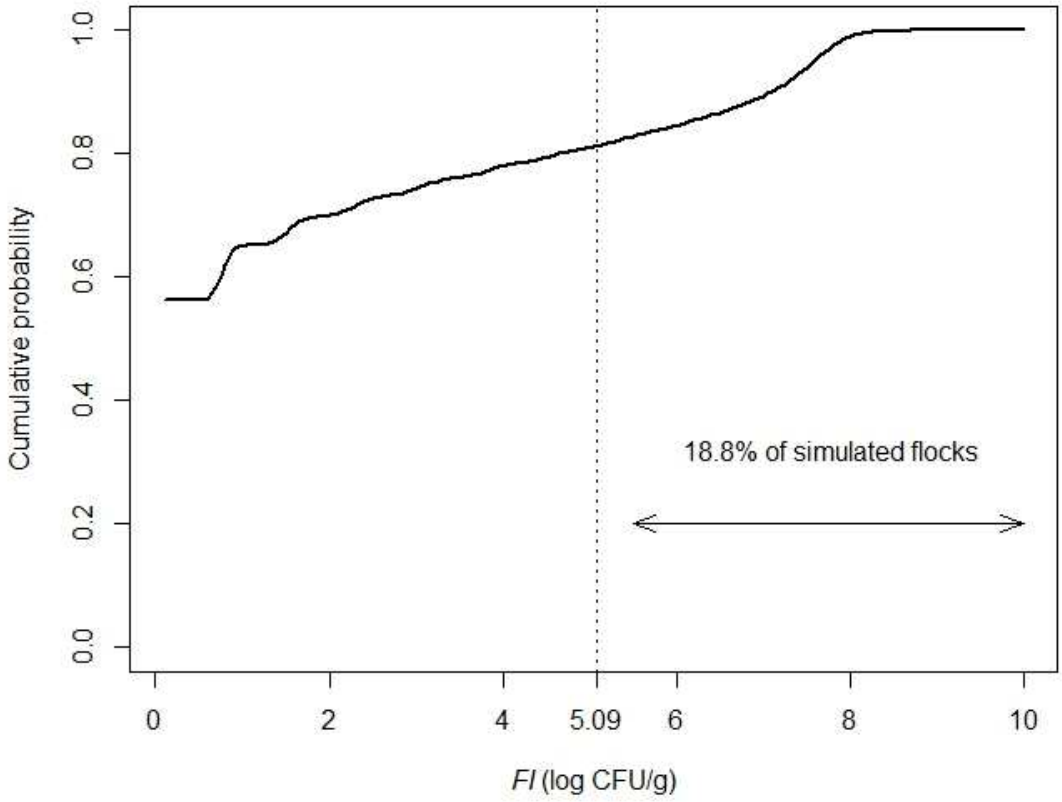
Figure 10 Tornado chart representing the model inputs ranked by effect on the output (Fl) mean. Each bar represents how much the respective input is able to displace the mean of Fl when all the others are fixed to theirs baseline values.

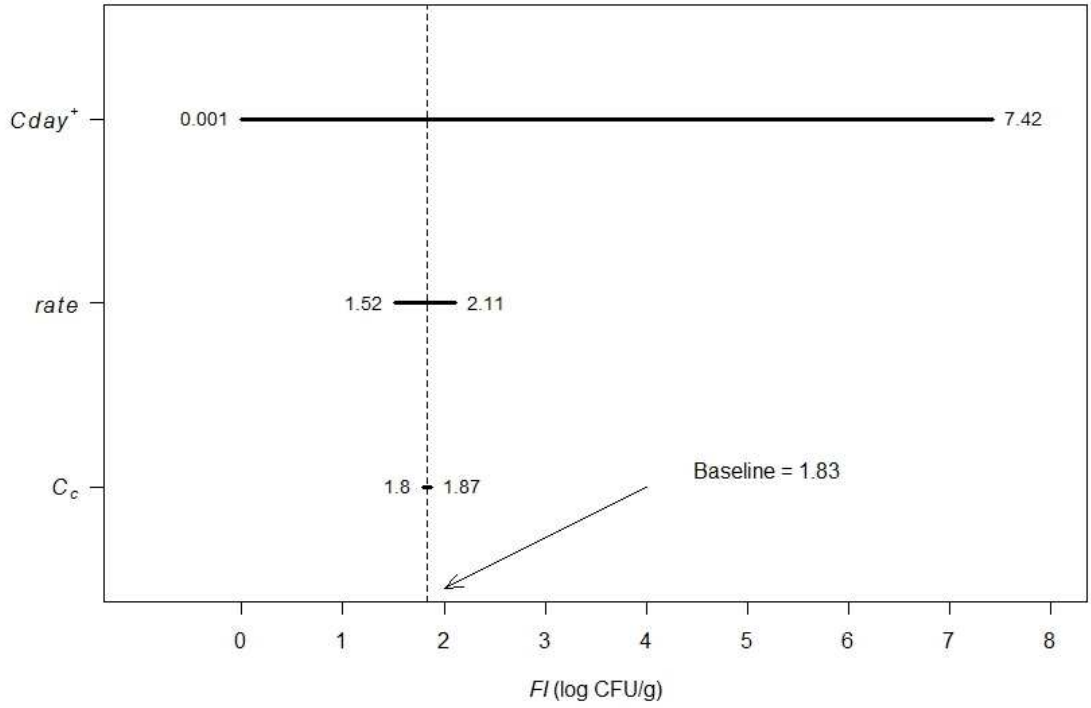

DAY OF INFECTION
MORTALITY RATE
POPULATION
SEEDERS
SPREAD WITHIN THE FLOCK
Mitigation strategies aimed at increasing resistance to colonization such as vaccines or feed additives.
WHITIN FLOCK PREVALENCE
Mitigation strategies aimed at reducing the microbial load in colonized animals such as bacteriophages or bacteriocins.
LEVEL OF CONTAMINATION
SAMPLES
FINAL LEVEL OF CONTAMINATION OF THE FLOCK AT CLEARANCE
BIOSECURITY
B+T-
B+T+
THINNING
B-T+
PROPORTION OF HIGHLY CONTAMINATED FLOCKS
B-T-

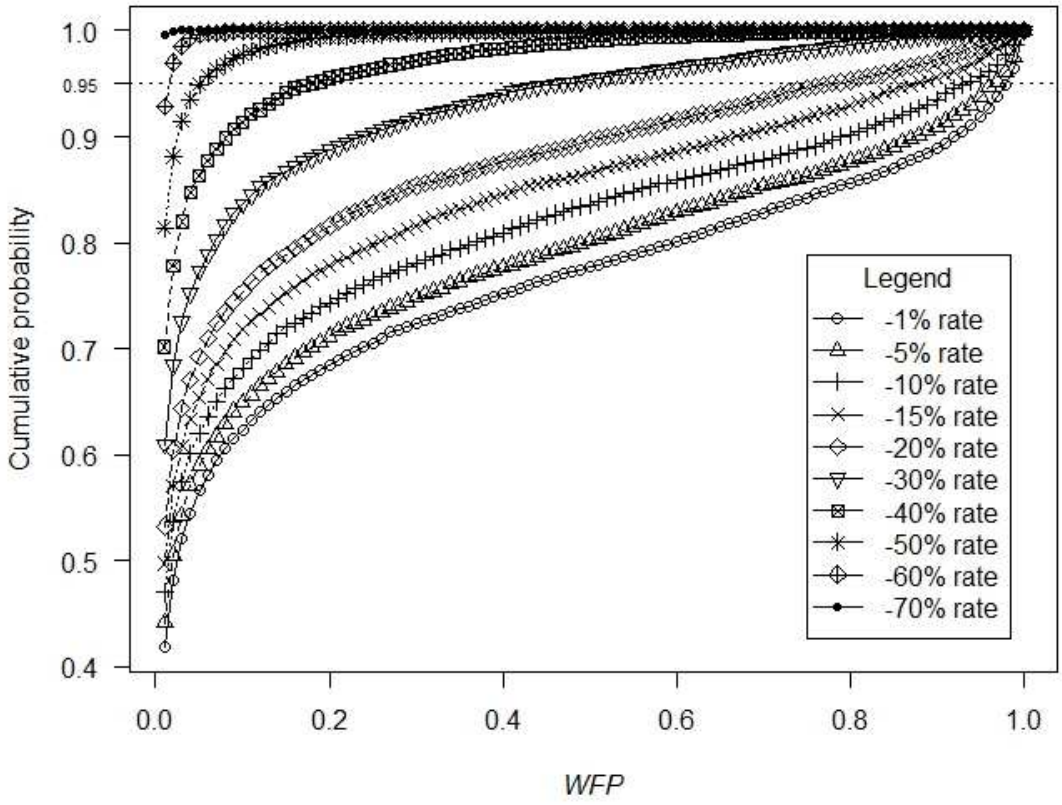

1.0
0.8
0.6
0.4
0.2
0.0
Cumulative probability
35.67
73.03
98.35
10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
Day of flock contamination

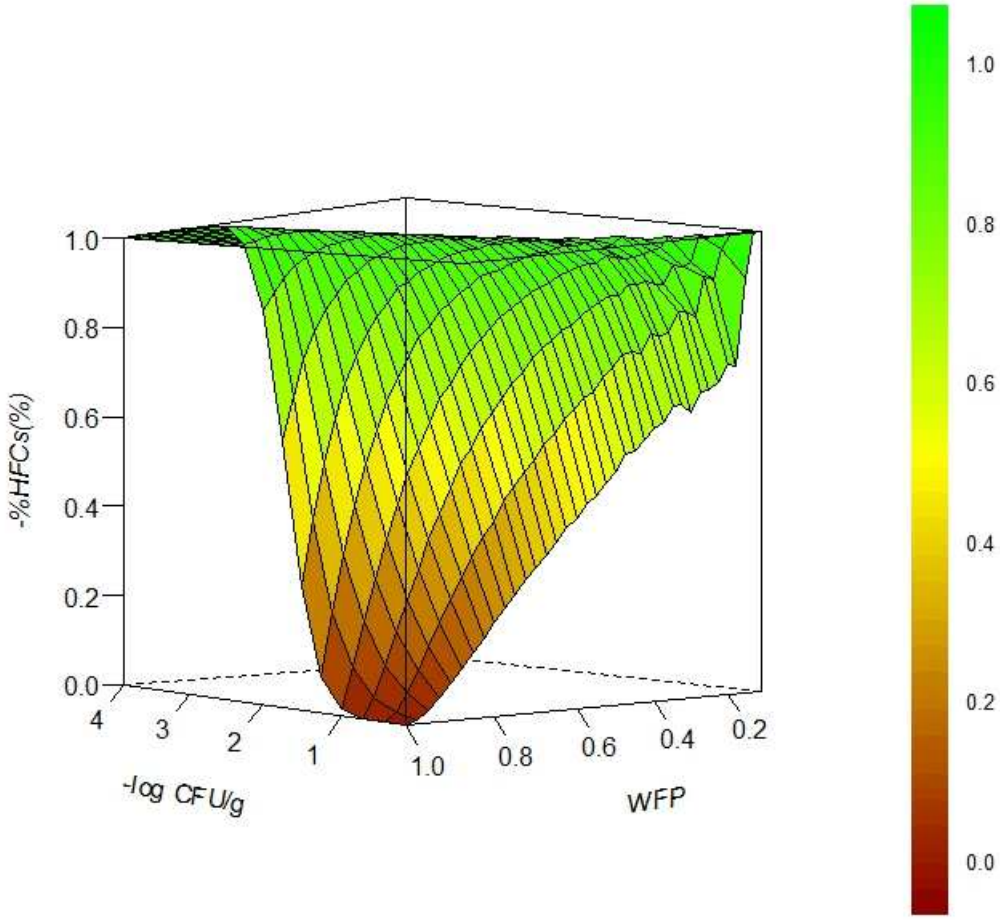

1.0
0.95
0.8
0.6
0.4
0.2
0.0
Cumulative probability
0 2 4 6 8 11 14 17 20 23 26 29 32 35 38 41 44 47 50
Day of infection

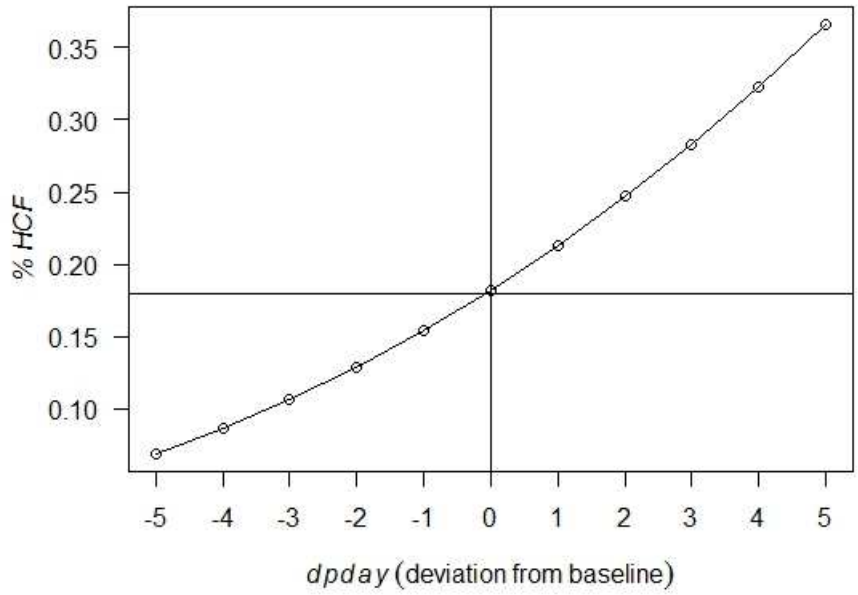

Frequency
0
10000
20000
30000
40000
50000
cumulative probability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.0
0.2
0.4
0.6
0.8
1.0
WFP

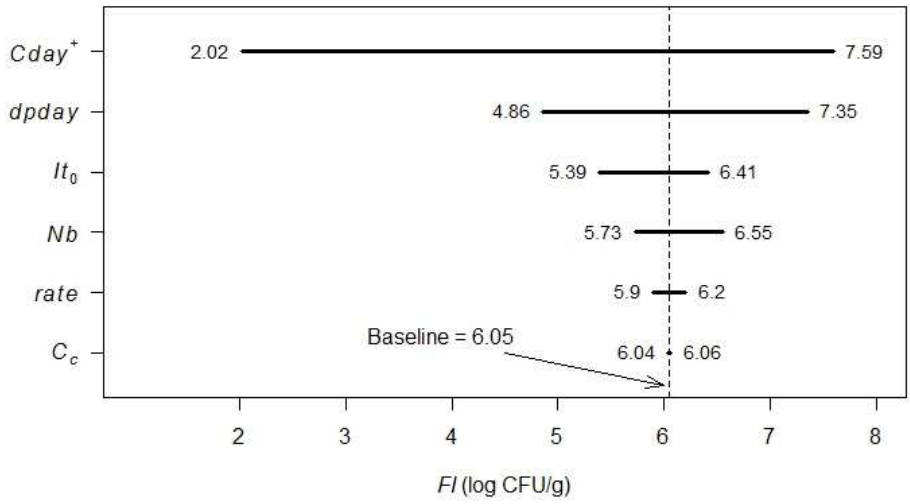

Cumulative probability
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
5.09
6
8
10
FI (log CFU/g)
18.8% of simulated flocks






1.0
0.8
0.6
0.4
0.2
0.0
-%HFCs(%)
4
3
2
1
-log CFU/g
1.0
0.8
0.6
0.4
0.2
WFP
1.0
0.8
0.6
0.4
0.2
0.0


0.35
0.30
0.25
0.20
0.15
0.10
% HCF
-5
-4
-3
-2
-1
0
1
2
3
4
5
dpday (deviation from baseline)



The effects of different options to control *Campylobacter* in chickens are quantified

The positive effect exerted by biosecurity could be thwarted if flocks are thinned

The final level of flocks' contamination is mainly related to the day of infection