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Presence of *Campylobacter* on Dutch broiler farms and associated risk factors

Report number WUR 1845095

Project team

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Summary

Campylobacter remains the most common reported zoonotic pathogen in humans in the European Union since 2008 (EFSA, 2018). Poultry is a major source of human infection with *Campylobacter*, although the epidemiology of *Campylobacter* at broiler farms is still poorly understood. The purpose of the study was to investigate factors associated with the presence of *Campylobacter* on a selected number of broiler farms in the Netherlands.

The investigations were conducted starting from spring/summer 2017 until autumn 2018. Faecal samples were collected on a weekly basis at 21 farms, from one, two or three houses. There were 38 houses participating in the study. Information on flock and farm characteristics including biosecurity measures was gathered via a questionnaire (CAMPAS) and food chain information forms (VKI forms). In addition activities (other than routine activities) at the farms were described by the farmers in log books. The collected data were used as putative variables for a risk factors analysis.

In total 284 flocks were sampled of which 32% were positive for *Campylobacter.* A multivariate logistic regression model revealed three factors associated with presence of *Campylobacter* in broiler flocks. They included summer/autumn season, mowing premises in the surroundings of the farm and the previous *Campylobacter* positive flock in the house. Although the factor breed did not remain in the final model, there was a difference in the time of becoming infected between regular and slow growing breeds. With the available data set the thinning practice was not confirmed as a risk factor.

Evaluation of the outcome of the biosecurity questionnaire (CAMPAS) revealed an association between both poor hygienic practices on farm as well as the number of houses on farms with *Campylobacter* presence. Some indicators addressed in the questionnaire however need adjustment to be used as a predictive tool for introduction of *Campylobacter* on broiler farms.

More studies are needed to further explore the role of the farm management practices on the *Campylobacter* presence in broiler flocks as well as the actual behaviour and compliance of farmers to biosecurity practices. The CAMPAS questionnaire will be further developed and validated to provide farmers with a tool to indicate the strengths and weaknesses in their biosecurity status and the associated risk for introduction of *Campylobacter* in their flocks.

1 Introduction

Campylobacter is the main cause of bacterial foodborne infections in the Netherlands and Europe. According to European Food Safety Authority 20-30% of *Campylobacter* infections in humans can be associated with consumption and/or (unhygienic) preparation of poultry meat; whereas up to 50-80% with poultry reservoir in general, via other transmission routes than consumption of poultry meat (EFSA, 2011). These possible routes include for example surface water, air or direct contact with poultry. Although poultry is thus known to be a major source, (inter)national efforts on the prevention of *Campylobacter* in poultry meat and meat products have had limited effect so far.

Wageningen University & Research collaborates with NEPLUVI (association of the Dutch poultry processing industry) and the primary poultry sector to reduce *Campylobacter* in chickens and meat, with the end goal to reduce the number of cases of campylobacteriosis in humans. The collaboration between research and practice in a Public Private Partnership under the Top-Sector policy of the Ministry of Agriculture, Nature and Food quality (AF-14203 'Beheersing van Campylobacter in de pluimveesector', BO-33.04 AF8) focuses on multiple lines of research.

This project aimed amongst other at investigation of *Campylobacter* presence and associated risk factors on 21 selected Dutch broiler farms. The study was carried out from spring/summer 2017 until autumn 2018. During this period the broiler houses were sampled on a weekly basis and farmers were encouraged to register activities (other than routine activities) performed on farm in a log book. The log books' records, the answers given by the farmers through a developed questionnaire on farm characteristics related to biosecurity in the form of a hygiene score 'CAMPAS' and together with information on individual flocks through the Food Chain Information forms ('VKI formulieren') were used as explanatory variables for the risk factor analysis.

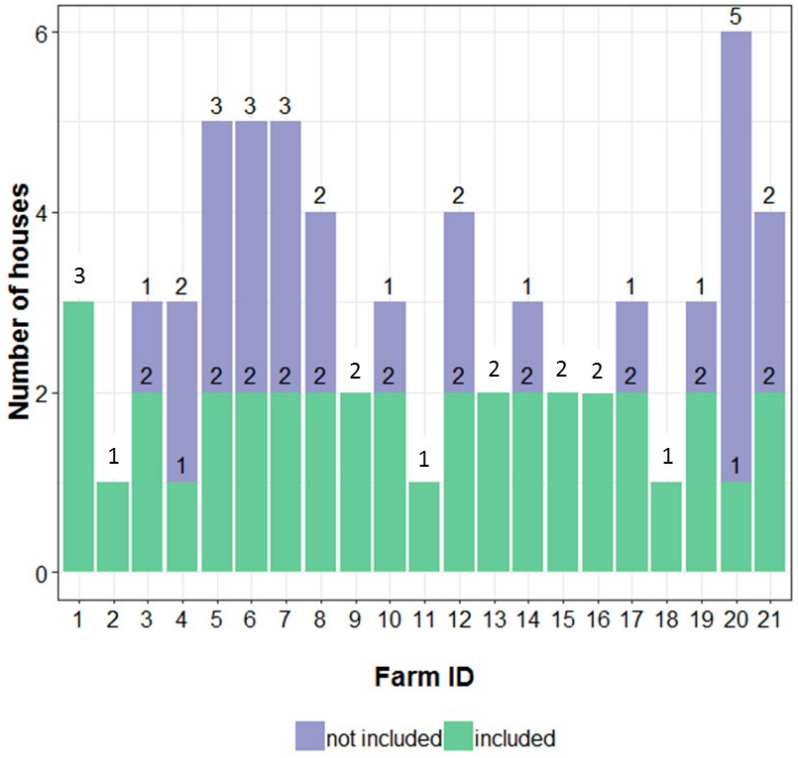
2 Materials/Methods

2.1 Study design

Investigated farms

Twenty one broiler farmers in the Netherlands volunteered to participate in the study. Inclusion of farmers was done based on convenience and the farmers were approached through the network of researchers. The study started in spring/summer 2017 and ended in autumn 2018. Per farm location a maximum of two broiler houses were included in the study. Some farmers only have one house, so they participated with one. One farmer participated with 3 houses, because of differences in design and biosecurity levels. Not always all houses of a farmer were included in the study and the total number of houses present on the farm varied from 1 to 6 per farm (Figure 1). In total there were 38 houses included in the study. At the start of the experimental investigations, the farmers were visited by members of the project team. All farmers had their own contact person (4 in total, 2 from WLR, 1 from WBVR, and a veterinarian from a veterinary clinic in the South of the Netherlands).

Figure 1. Number of houses participating in the study (green) and total number of houses per farm (sum of green and purple).



Data collection by CAMPAS questionnaire

For each participating farm a questionnaire (CAMPAS, i.e. *Campylobacter* compass) was filled in, together with the contact person from the project team. This questionnaire was based on the national biosecurity check questionnaire (IKB hygiënescan, https://www.avined.nl/thema/bedrijfshygiene), but modified in some aspects to make it more specific for *Campylobacter*. Questions were related to six categories: the surroundings of the farm, the farm premises, farm hygiene, hygiene in the broiler house, materials and vehicles, and pest control. The CAMPAS questionnaire is included in the Appendix 6.1.

With respect to the CAMPAS this study aimed at evaluating whether the outcome of the CAMPAS questionnaire corresponds with the results on the *Campylobacter* presence in broiler flocks on the participating farms.

Collection of samples
During the first visit, the contact person informed the farmers on the purpose of the study and gave an explanation about the sampling procedure and administration. Farmers were asked to collect weekly a faecal sample from each participating house, starting in general from the second week after arrival of the chickens into the house until slaughter of the flock. Faecal samples were collected in a plastic sample container, by walking through the house and picking up fresh faecal material from 4-6 different locations in the house. The faecal samples were labelled and stored in a -20°C freezer provided by the project team until the end of the cycle. Then they were collected by the assigned contact person and delivered to the National Reference Laboratory for *Campylobacter* at WBVR in Lelystad.

Data collection by log books
Furthermore, the farmers were asked to fill in the log books. These log books gathered information about activities that took place in the house (should be filled in separately per each participating house in the study), and an extra log book for activities on/around the farm (so 3 parts of log book in total, when 2 houses were participating; sometimes the farmers combined the log books for the 2 houses, because activities were the same). The date(s) for each specific activity were registered in the log books. Routine activities, like for example making the daily round in the house, feeding, were not written down. The list of the log books' activities is presented in the Appendix 6.2. Next to activities listed in the log books the farmers were free to add other activities.

Fly traps
During the summer months the farmers also hung up the "fly traps" (sticky papers on which flies will be caught when landing on it; Silvalure fly paper for stables, 585x300 mm, Silvandersson Sweden AB) in the broiler house, and for example in the front room ("voorlokaal"), to check whether flies were present.

Delivery of samples and data
After each cycle, i.e. delivery of broilers to the slaughterhouse, a project team member visited the farmer to collect the samples and evaluate the cycle. Short interviews were conducted to discuss occurrence of non-standard activities and potential health issues of the flock.
The project team member collected per each cycle: faecal samples (including the form with start and slaughter data and sampling data), fly traps, log books, VKI forms (VKI = food chain information), and if possible "stalkaarten". The protocol for collection of faecal samples and submission form is included in the Appendix 6.3.

2.2 Handling of samples and information

CAMPAS questionnaire scores
The CAMPAS questionnaire consisted of 86 questions divided in 6 categories, i.e. Surroundings (Omgeving), Farm premises (Bedrijfsterrein), Farm hygiene (Bedrijfshygiene), House hygiene (Stalhygiene), Materials and vehicles (Materialen en voertuigen), Pest control (Ongediertewering-en bestrijding). The questions can be answered by yes or no. Per question, a score of 0 was given if not applicable (no) or 1 if applicable (yes) (Appendix 6.1). In some cases, a score of 0.5 was given, for example when the question was like "Maakt iedereen op het bedrijf gebruik van bedrijfseigen kleding?", and this was done for visitors, but not for the farmer himself.

The CAMPAS scores were calculated per each category as follows: *10 x (sum of answers per category/total number of questions per category).* Adding the scores of the individual categories resulted in an total CAMPAS score ranging from 0 to 60 per farm.
A lower score indicated either higher biosecurity measures or lack of the presence of potential sources of *Campylobacter* on or around the farm and potentially a lower risk for *Campylobacter* introduction in the chicken house.

Laboratory methods
Submitted faecal samples were analysed at the National Reference Laboratory for *Campylobacter* at WBVR using a real-time PCR (Josefsen et al., 2004).

Fly traps were scored for the amount of flies: i.e. many, intermediate or low. Then they were stored in the fridge until results from the faecal samples were available. In case of positive results, the flies were also tested for *Campylobacter* presence by real-time PCR.

Data base
Selected information obtained from the CAMPAS, log books and VKI forms was gathered in a data base. This data base was used for selection of variables for the risk factor analysis.

2.3 Data analysis

***Campylobacter* positive flocks**
The number of positive flocks to total number of flocks sampled on the farms included in the study during 2017 and 2018 was visualised per month of the year (pooling both years' data), per farm and per house on a farm.

Explanatory variables
Information collected by various sources, i.e. CAMPAS questionnaire, log books and food chain information forms (VKIs) were used as explanatory variables for risk factor analysis. Explanatory plots of a particular variable versus *Campylobacter* status per flock (positive and negative) were visualised and are summarised in Appendix 6.4.

For numeric variables the Pearson's correlation was calculated in order to eliminate correlated variables prior to the risk factor analysis (VKI variables and CAMPAS scores). Correlation coefficient above 0.5 indicated correlated variables. In such case only one variable was used as an explanatory variable in the risk factor analysis. For categorical ones the Chi-square was calculated.

Fly traps
Fly traps were photographed. The number of flies on the trap was classified in following categories: high (trap full of flies/insects), low (<10 flies) and intermediate (in-between high and low). Correlation between amount of flies and *Campylobacter* status in the faecal samples was checked by Chi-square test.

Risk factors
Two separate models were tested, one included explanatory variables from VKI-forms, log books and farm characteristics. The second one included variables from the CAMPAS scores together with farms characteristics. The second model was done to better estimate the value of CAMPAS scores as predictor for the flocks to become *Campylobacter* positive. The methods of the analyses were the same, only the variables included differed.

In the first model the flock was the unit of analysis and its status (*Campylobacter* positive/negative) was the response variable. The generalised linear mixed effect models (GLMM) were fitted. The available data had a hierarchical structure where flock is a nested factor in a house and this is nested in a farm. To account for dependence of flock related information within a farm, the random effect for farm was introduced. Also house was tested as an additional random effect clustered in the farm, but adding it did not improve the model fit. Hierarchical structure of random effects, i.e. farm and house did not add to explanation of variance in the model, thus the factor house was excluded.

Firstly the univariate analysis was done, including variables selected from the VKI forms, and the CAMPAS questionnaire (only on farm characteristics, not the CAMPAS scores): location of the farm, breed, age, season, daily mortality, *Salmonella* status, thinning, presence of diseases, type of side activity, number of houses on farm, number of chickens per house, number of broilers on the farm, status of the previous

cycle. The explanatory variables were modelled as fixed effects whereas a farm as a random effect. Each model with a fixed explanatory variable and random variable was compared to a null model without the explanatory variable, having only the random variable (farm). These models were nested (that means that a null model was a simplification of a model with an explanatory variable) and fitted using the same number of observations. They were compared by the likelihood ratio test (LRT) to determine the significance of an explanatory variable. The explanatory variables with p value (from the LRT) <0.25 were selected for the multivariate analysis.

In addition as a univariate analysis of variables from the log books each activity described in the log books was analysed with *Campylobacter* status of the flocks by the Chi-square or Fisher's exact test. Variables with p<0.05 of the test were considered as associated with the *Campylobacter* status and thus selected for following multivariate analysis. Variables with p>0.05 were not included for the risk factor analysis, since association between a variable and the *Campylobacter* status could not be concluded.

Firstly a multivariate model with all variables (having LRT p value <0.25, or Chi-square or Fisher's exact test <0.05 in case of the log books variables) was fitted. Afterwards variables were eliminated by backwards selection from the one with the highest p value (based on the Wald's test) to the lowest. After eliminating a variable the models were evaluated by the Akaike's Information Criteria (AIC). A model with the lower AIC criterion was selected for the next step until removing further variables did not improve the model anymore, i.e. did not reduce the AIC value further. The model fit of the final model was assessed by evaluating the residuals.

The CAMPAS biosecurity scores and farm characteristics were modelled in a separate second model in order to evaluate whether the scores can be used to indicate farms having higher chance of becoming colonized with *Campylobacter*. In this model the proportion of positive flocks per farm was the response variable. The explanatory variables included the CAMPAS scores and variables on farm characteristics: location of the farm, breed, number of houses on farm, type of side activity, number of broilers on the farm were included. As in the first model first the univariate selection of variables was done and variables with p value (from the LRT) were selected for the multivariate analysis, performed as described above.

The data analysis was performed in the statistical package lme4 of the R software, version 3.5.0 (R Development Core Team, 2018). Variables in the final models with a p-value below 0.05 were considered as significant. Their coefficients were presented as exponential value to estimate the odds ratio and the 95% confidence interval.

3 Results

3.1 *Campylobacter* presence on studied farms

In total there were 284 flocks sampled at the 21 farms, of which 90 were positive for *Campylobacter* and 194 negative. The first samples were collected in June 2017 and the last in November 2018. Not all farmers participated with the same intensity in the study, that means the number of flocks sampled was not equal per farm. This is related to the lengths of cycle and downtime at a particular farm. One farmer (Nr. 4) withdrew from the study after three cycles. The number of cycles sampled per farm is presented in Table 1 below.

Table 1. Summary of number of cycles sampled per farmer

FarmerID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Number of cycles	7	9	7	3	5	4	9	6	9	9	7	8	8	8	10	7	11	8	6	7	8

As visualised in Figures 2 - 4 there were two farms that remained negative through the entire study. The rest of the studied farms were at least once positive in at least one house.

Figure 2. Location of the studied farms in the Netherlands and their *Campylobacter* status during the study (June 2017 until November 2018). Blue dots indicate negative farms whereas yellow farms tested positive at least once during the study period.

Figure 3 presents the number of positive and negative flocks per each studied house. Farm 11 and 18 (having only one house) remained negative through the entire study, while the rest of farms had both positive and negative results.

Figure 3. Number of positive (blue) and negative (green) flocks per house. The first digits in the numbers represent the farm ID, while the second digit represents a particular house of that farm, i.e. the number 1.2. identifies the second house of farm number one.

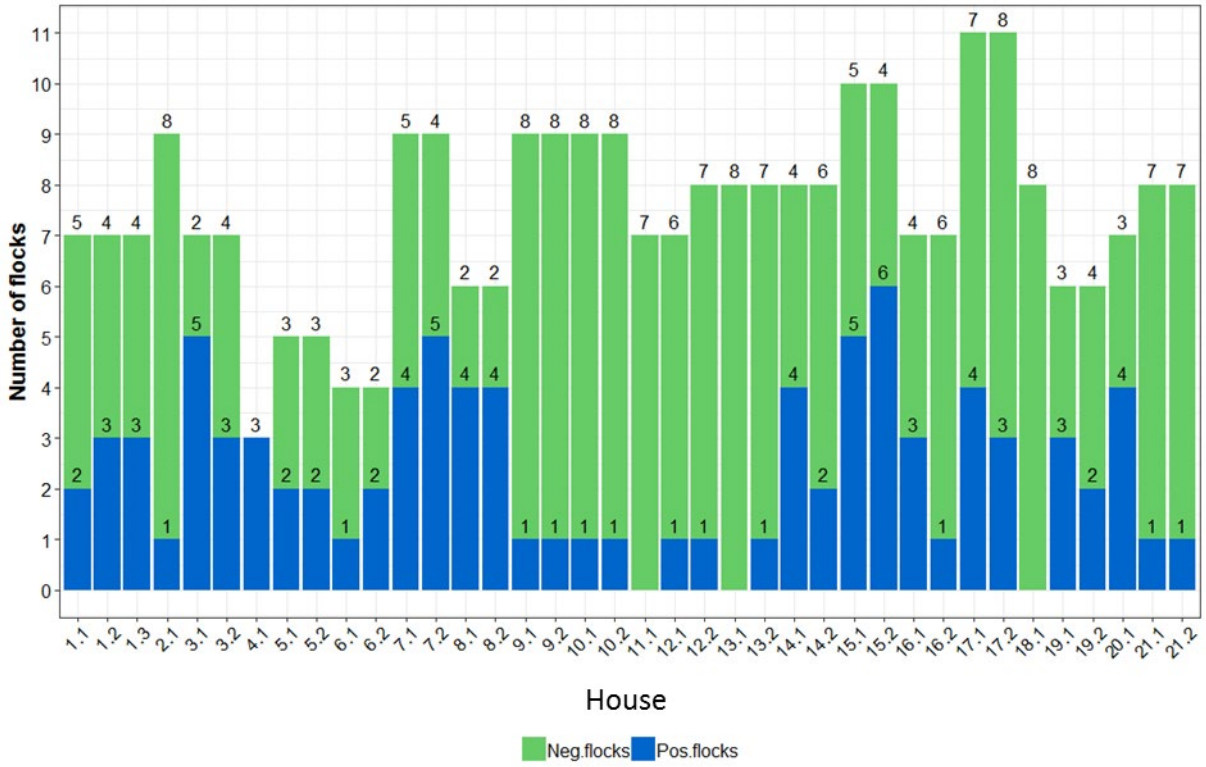
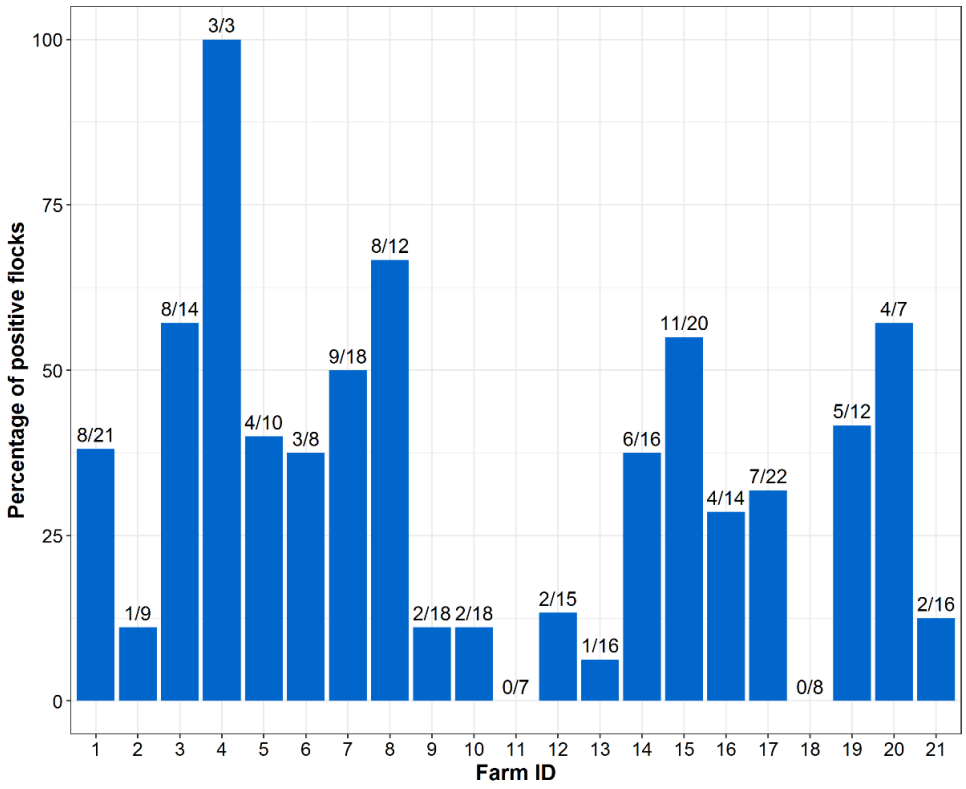
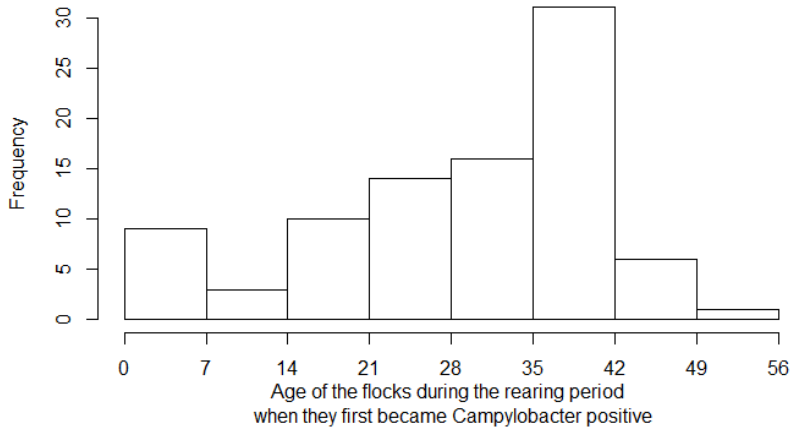


Figure 4. Percentage of positive flocks per farm. Numbers above the bars show the number of positive flocks/out of number of sampled flocks on particular farm.



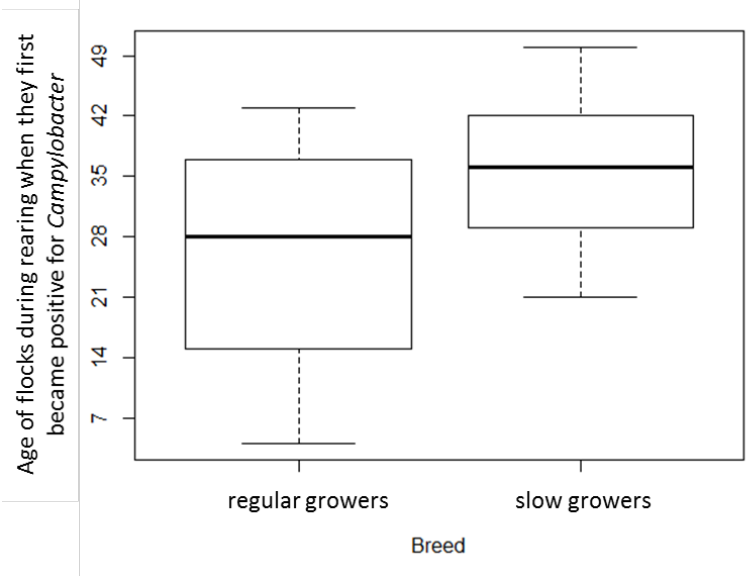
The samples were collected every week. Figure 5 below summarises the day (age) during rearing when the flock first became positive for *Campylobacter*. This day was calculated based on the date of placing the broilers in a house and date of detecting the first positive sample in a flock. These results show that the majority of flock became positive between day 21 and 42, what corresponds to 76% of all positive flocks (so 68 out of 90 positive in the entire study). These results might be overestimated, since the samples were not collected every day, but once per week.

Figure 5. Frequency of positive flock at the age of the flock during the rearing period when they first became *Campylobacter* positive. Data from 90 broiler flocks sampled at 21 broiler farms in the period June 2017- November 2018.



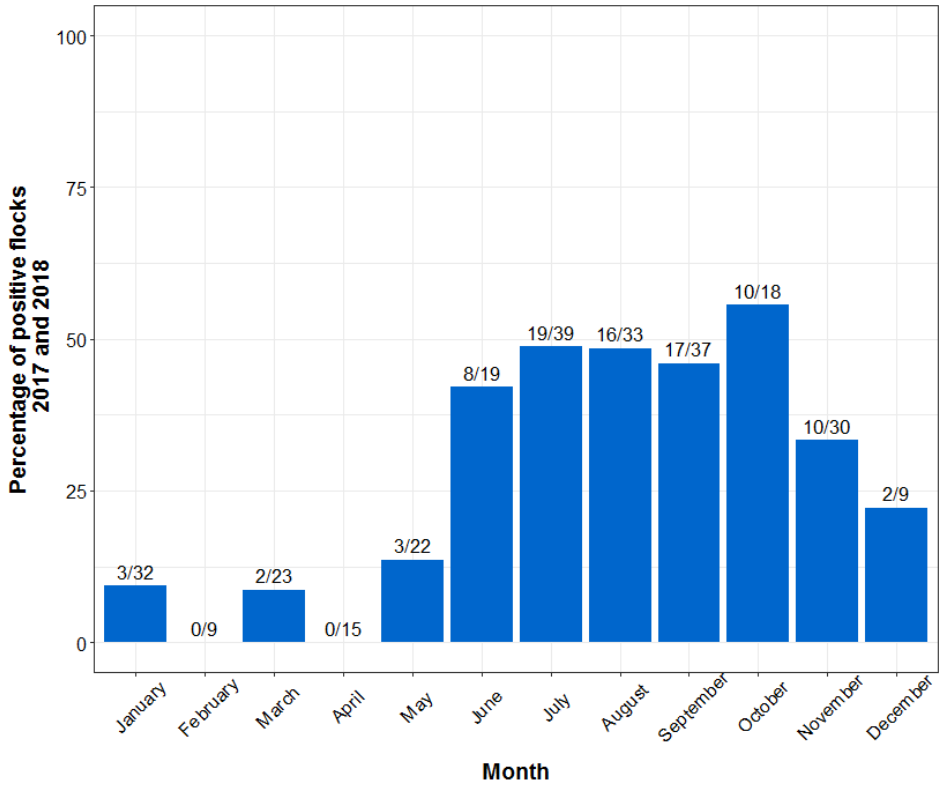
The Figure 6 summarises the age at which the flocks of regular broiler breeds and slower grower breeds became positive. Based on the linear regression the age of becoming infected was lower for regular breeds by 12 days (CI 5;18) compared to slow growing breeds ($p<0.01$). On average the regular growers became infected at the 26th day, whereas the slower growing at the 38th day. Again, these results might be overestimated, since the samples were not collected every day, but once per week.

Figure 6. The age of becoming *Campylobacter* positive divided per regular and slow growing breeds. Data from 90 broiler flocks sampled at 21 broiler farms in the period June 2017- November 2018. The length of the boxplot indicates the interquartile range (IQR) of the data (50% of the data), the horizontal bar inside the boxes indicates the median value; whiskers represent 1.5 × IQR or the maximum/minimum value of the dataset.



The seasonal variation in *Campylobacter* presence on the studied farms is summarised in Figure 7 and presents pooled data per month combining both years 2017 and 2018. It shows an increase in the presence from June until November and decrease from December until May. The average percentage of positive flocks in the entire study was 32%. This trend corresponds with findings reported earlier in the Netherlands (Anonymous, 2018).

Figure 7. Percentage of *Campylobacter* positive flocks per month at slaughter age at 21 Dutch broiler farms (data collected in the period June 2017 to November 2018).



3.2 Explanatory variables on flocks and farm characteristics

Descriptive information on explanatory variables gathered with the help of VKIs on flock level are summarised in Table 2. For 31 flocks these forms were unavailable what resulted in missing values and reduction of the data set for the risk factors analysis. Out of 284 studied flocks in the entire study 209 were included in the risk factor analysis due to excluding flocks with missing variables.
Explanatory plots of the flock related variables versus the *Campylobacter* status of analysed flocks are summarised in the Appendix 6.4. The information on farm characteristics collected by CAMPAS questionnaire are also included in Table 2.

Season was included as an explanatory variable with two levels, i.e. summer/autumn including June – November and winter/spring including December - May.

Status of the previous flock in a house was introduced as an explanatory variable. This information was however missing for the first sampled flocks i.e. 36 observations. For these flocks the status was estimated by so called nearest neighbour imputation algorithm. In fact the *Campylobacter* results of the first sampled flocks was used to indicate the status of the previous one for this 36 observations.

Results of the scores obtained by the CAMPAS questionnaire including Surroundings (Omgeving), Farm premises (Bedrijfsterrein), Farm hygiene (Bedrijfshygiene), House hygiene (Stalhygiene), Materials and vehicles (Materialen en voertuigen), Pest control (Ongediertewering-en bestrijding) are shown below in

Figure 8. The total score is omitted since the scores per particular category are more informative. The results of the scores are plotted against the percentage of positive flocks on farms. This figure shows that no clear trend can be observed between the scores (on biosecurity measures) and the percentage of positive flocks.

To determine related variables the correlation was checked. The Pearson's correlation coefficient between the scores obtained by the CAMPAS questionnaire revealed correlation coefficient above 0.6 for Farm premises (Bedrijfsterrein) and House hygiene (Stalhygiene) and for Farm hygiene (Bedrijfshygiene) and House hygiene (Stalhygiene). A correlation was also examined between numeric variables on farm characteristics and between the scores obtained by the CAMPAS questionnaire. A Pearson's correlation coefficient of 0.56 was found between the variables "number of broilers on farm" and "number of houses per farm".

Figure 8. Results from the CAMPAS questionnaire. The higher the score the more risky situation is assumed i.e. higher score was expected to indicate that *Campylobacter* introduction might be more likely. The scores are plotted against the percentage of positive flocks on farms.

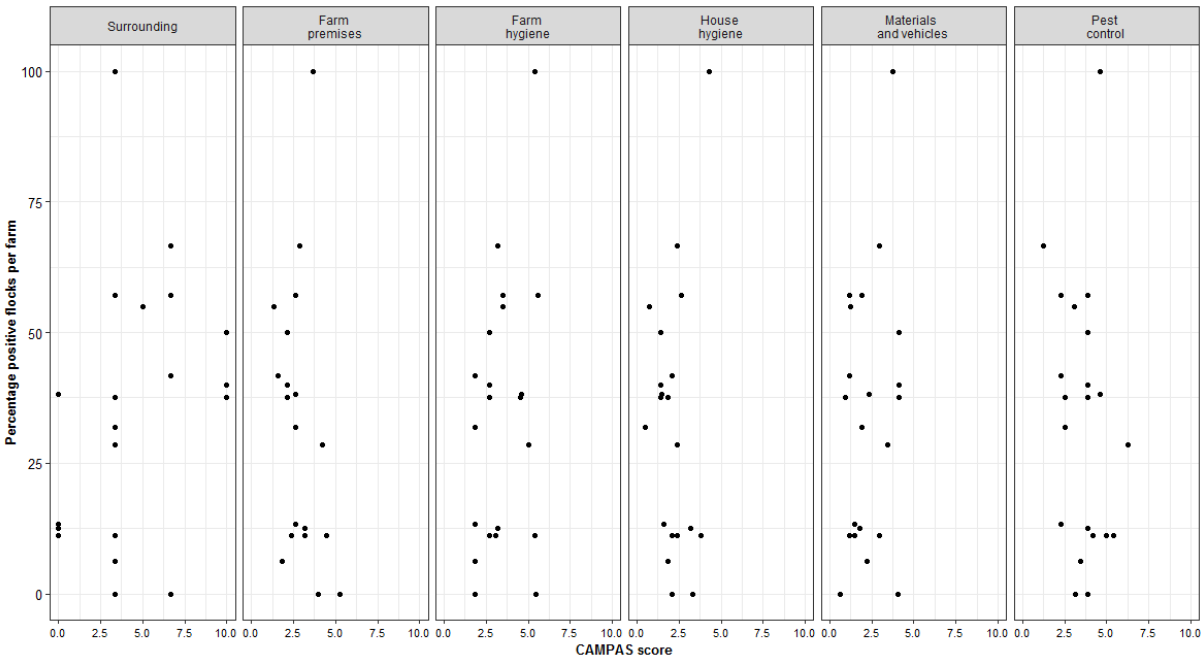


Table 2. Descriptive statistics of the explanatory variables gathered by the VKIs and the CAMPAS questionnaire based on 284 flocks, originating at 21 farms.

Explanatory variables	Response	Frequency [number of flocks/farms]	Number of missing values	Mean	SD	Min	Max
Obtained from VKI forms on flock level							
Number of rounds				7	2	3	11
Location	East	139					
	North	50					
	South	95					
Status of the previous flock	Positive	94					
	Negative	190					
Breed			30				
	Slower growing i.e:	162					
	Hubbard JA987	69					
	Hubbard 757	53					
	Aviagen lines#	40					
	Regular growing i.e.	92					
	Cobb	5					
	Cobb/Ross	4					
	Ross 308	83					
Age [days]			17	47	7	29	63
Daily mortality [%]			34	3.1	2.0	0.3	14
Season*	Summer/autumn	176					
	Winter/spring	108					
Diseases			33				
	yes	51					
	no	200					
Thinning			5				
	yes	92					
	no	187					
Salmonella status			38				
	positive	22					
	negative	224					
Obtained from CAMPAS on farm level							
Type of side activity	none	11 farms					
	other than animals	5 farms					
	with animals	5 farms					
Number of broilers on farm				74664	38684	9500	150000
Number of houses per farm				3	1	1	6
Number of broilers per house				25284	11291	5940	53000

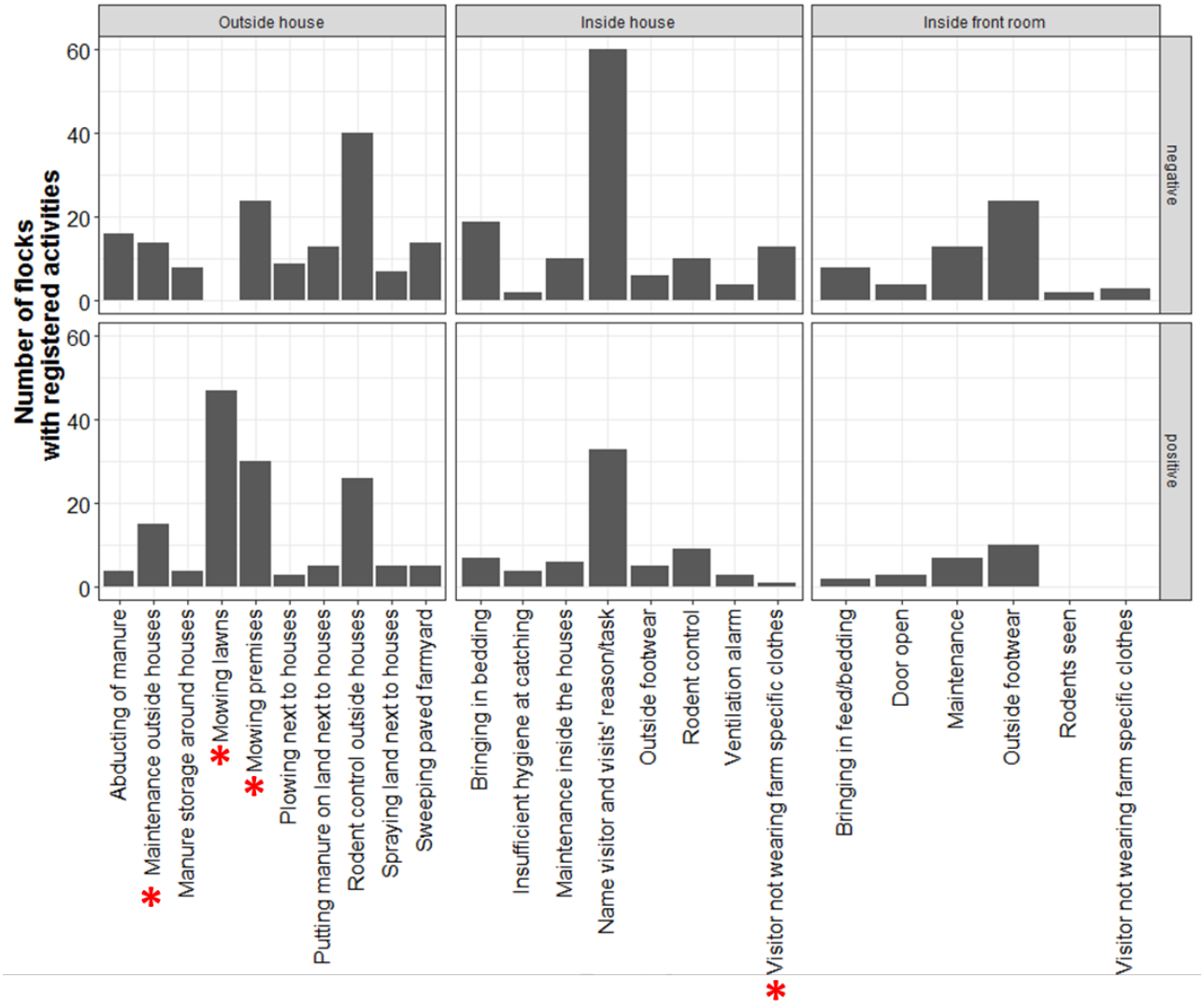
*where summer/autumn includes following months June-November; whereas winter/spring includes December-May.

includes Gold Ranger (1 flock), Ross Rowan (2 flocks), Rowan Ranger (3 flocks), RRR (34 flocks)

3.3 Results from the log books

Out of the 284 sampled flocks, log books were available from 243 flocks. Figure 9 summarises the activities monitored through the log books versus the status of the flocks (positive/negative for *Campylobacter*). Not always the log books were completed what resulted in a reduction of the available data set for the risk factors analysis. Based on the Chi-square or Fisher's exact test used as a univariate analysis of these variables a number of activities were determined to be associated with the *Campylobacter* status. Those included mowing of the lawns (p-value = 0.006), mowing of the premises (p-value <0.01), maintenance outside the houses (p-value =0.03) and visitor in the house not wearing farm specific clothes (protective overall provided by the farmer) (p-value =0.04). These variables continued to the multivariate analysis.

Figure 9. Activities reported in the log books versus status of the flocks for which the activities were performed. Asterisks indicate the activities with significant results from the univariate analysis (Chi-square or Fisher's exact test) indicating an association between the activity (variable) and the *Campylobacter* status.



3.4 Results fly traps

From 65 analysed fly traps the majority contained a low (40) or intermediate (14) number of flies. A large variety of different types of flies and other insects were present on the traps: normal house flies, but also mosquitoes, culicoides, small flies (fruit flies) and very large mosquitoes. No correlation was found between number of flies and positive faecal samples for that round.

3.5 Risk factor analysis

Two separate models were tested, one included variables from VKI-forms, log books and farm characteristics. Variables from the CAMPAS scores together with farms characteristics were included in a second model. This was done to better estimate the value of CAMPAS scores as predictor for the flocks to become *Campylobacter* positive. The methods of the analyses were the same, only the variables included differed.

In the first model, based on information from VKI forms, and farm characteristics, out of 13 variables that fitted in the univariate models there were 7 variables with LRT p value below 0.25 (indicated by hashtag (#) in Table 3). This is an arbitrary threshold and recommended by Hosmer and Lemeshow (Hosmer, 2000).

Table 3. Results of the univariate analysis and variables with p<0.25 from the LRT are indicated by hashtag (#).

Variable	Estimate (beta)	SR	p value Wald	p value LRT
Location	-1.69	0.65	0.01	0.01#
Breed	-0.69	0.48	0.15	0.15#
Season	-2.46	0.48	0.00	<0.01#
Age	-0.02	0.04	0.63	0.63
Daily mortality	0.17	0.09	0.06	0.06#
Salmonella status	0.09	0.74	0.91	0.91
Thinning	0.48	0.45	0.28	0.29
Diseases	0.07	0.43	0.87	0.87
Status previous flock	-2.05	0.33	0.00	<0.01#
Side activity	-1.89	0.48	0.00	<0.01#
Number of houses	0.43	0.21	0.04	0.03#
Number of broilers on farm	0.23	0.68	0.74	0.25
Number of chickens per house	0.38	0.51	0.45	0.45

Out of the 7 variables 6 were included in the multivariate analysis. Side activity was not included since smaller farms (with less houses) in general performed side activity, therefore the number of houses was used instead to avoid collinearity (Appendix 6.5). In addition four variables from the log books (as described in 3.3) were selected for the multivariate analysis

The multivariate logistic regression revealed risk factors associated with presence of *Campylobacter* after elimination of the variables backwards. The following three variables remained in the final model: season, mowing of the premises, status of the previous flock. Table 4 summarised the results of the multivariate model.

The odds of *Campylobacter* presence in a flock increased in summer/autumn as compared to winter/spring (OR=6.03, p<0.01), increased when premises nearby were mowed (OR=2.77, p=0.01) compared to flocks where the premises were not mowed, increased when the previous flock was positive (OR=6.35, p<0.01) compared to flocks with negative flocks in proceeding cycle.

Table 4. Variables included in the final multivariable logistic regression model on factors associated with the presence of *Campylobacter* in Dutch broiler flocks (209) sampled on 21 selected farms, in 2017 and 2018.

Explanatory variable	Number of flocks			Estimate	SE	OR	95% CI		p value
	all	*Campylobacter*		beta	beta		2.5%	97.5%	Wald test
		positive	negative						
Intercept				-1.22	0.49	0.30	0.10	0.69	0.01
Season									
Summer/autumn	132	63	69	1.80	0.48	6.03	2.50	16.91	<0.01
Winter/spring	77	7	70						
Status previous flock									
positive	76	47	29	1.85	0.37	6.35	3.04	13.15	<0.01
negative	133	23	110						
Mowing premises									
no	162	41	121						
yes	47	29	18	1.02	0.41	2.77	1.25	6.44	0.01

The second model, for the evaluation of the CAMPAS questionnaire, revealed significant association between increase in *Campylobacter* presence on farms and number of houses (OR=1.49, p<0.01), higher score for farm hygiene (indicating poorer biosecurity measures) (OR=1.25, p<0.01), whereas higher scores of pest control (indicating poorer pest control measures) and farm premises (indicating poorer biosecurity measures at the farm premises or the presence of potential sources of *Campylobacter* on the farm) were associated with more negative flocks (OR=0.73, p=0.04; OR=0.86, p<0.01 respectively) (Table 5). These two indicators might be confounded by unknown factors that were not measured during the current study. This points to the need to further adjust the CAMPAS questionnaire and potentially revise some questions, clarify their interpretation and/or merge questions into fewer categories.

Table 5. Results of the multivariate logistic regression model for evaluation of the presence of association between the scores on biosecurity measures obtained by the CAMPAS questionnaire.

Explanatory variable	Estimate	SE	OR	95% CI		p value
	beta	beta		2.5%	97.5%	Wald test
Intercept	-1.07	0.92	0.34	0.04	1.89	0.24
Number of houses	0.40	0.13	1.49	1.15	2.01	<0.01
Farm hygiene	0.49	0.14	1.63	1.25	2.24	<0.01
Pest control	-0.32	0.15	0.73	0.53	1.00	0.04
Farm premises	-0.60	0.22	0.55	0.34	0.86	<0.01

4 Discussion and conclusions

Campylobacter remains the most common reported zoonotic pathogen in humans in the European Union since 2008 (EFSA, 2018). Poultry remains a major source of human infection with *Campylobacter*, although the epidemiology of *Campylobacter* at broilers farms is still poorly understood (Ridley, 2011).

The purpose of the current study was to investigate the presence of *Campylobacter* on 21 broiler farms in the Netherlands and to determine factors associated with the presence in broiler flocks. Inclusion of farmers was done based on convenience and the farmers were approached through the network of researchers. Information on flock and farm characteristics were gathered via various questionnaires and faecal samples were collected per each flock on a weekly basis from one, two or three houses at the participating farms. The study actively involved selected farmers in monitoring of various activities that could have been associated with the presence of *Campylobacter* in the flocks.

In total the results of 284 flocks sampled at 21 Dutch farms revealed the presence of *Campylobacter* in 32% of the flocks through the entire study (from spring/summer 2017 until autumn 2018). This corresponds with the results published in the Netherlands earlier (Anonymous, 2018; Bouwknegt et al., 2004).

Further, the final multivariate logistic regression model using input from the log books, the VKIs and information on characteristics of the farms revealed three factors associated with presence of *Campylobacter* in broiler flocks, including summer/autumn season, mowing premises in the surrounding of the farm and the previous *Campylobacter* positive flock in the house.

The association of the summer/autumn season with an increase in *Campylobacter* presence in poultry flocks is in agreement with previous studies (Bouwknegt et al., 2004; EFSA, 2011). In the current study, we observed an increase in the presence of *Campylobacter* starting in June and lasting until November and decreasing between December and May, in line with national data sources (Anonymous, 2018).

Another factor associated with the presence of *Campylobacter* was related to mowing of the premises in the farm surrounding. This activities were performed both in the summer/autumn and the winter/spring season what may explain that next to the factor of season this variable remains in the model. In the Chi square test the mowing and season revealed to be associated ($p<0.05$), however removing the mowing variable from the model decreased the model fit. Mowing of the vegetation was also determined in a univariate analysis while investigating risk factors associated with high pathogenic avian influenza (HPAI) infection on the laying hens farm (Garber et al., 2016). Reports on practices to prevent introduction of high and low pathogenic avian influenza to poultry houses in the Netherlands argue for limiting the mowing of the vegetation around the houses, since it might lead to virus introduction from the environment via ventilation (Bokma, 2016). With respect to *Campylobacter* it is recognised that it is present in the surroundings of the broiler farms (EFSA, 2011). Mowing and variables that might be related to mowing shall be thus further explored.

Further an association was found between *Campylobacter* presence in a flock and a previous *Campylobacter* positive flock in the house. Various studies indicated limited effect of the carry-over of *Campylobacter* from one flock to a subsequent one in the same house (EFSA, 2011). *Campylobacter* is widely spread in the environment surrounding the house and its reintroduction from the environment may play a role (Hiett K.L., 2002). This role is supported by the study where different *Campylobacter* serotypes in the subsequent cycles in the broiler houses were detected (Jacobs-Reitsma W.F., 1995). Thus this finding may indicate that certain farms are at higher risk for transmission of *Campylobacter* in their houses and therefore have a higher probability of subsequent flocks becoming colonized.

Broiler production has changed as a result of the consumer demand to purchase breeds produced under higher welfare standards. Therefore next to the regular growing breeds also slower growing breeds are produced. The majority (76%) of the *Campylobacter* positive flocks in this study became positive between

21 and 42 days of age. It was observed that on average the regular growing breeds became positive at the 26th day, whereas the slower growing breeds at the 38th day. These results might be overestimated, since the samples were not collected every day, but once per week. It is not clear what causes this difference. Factors that may play a role are that slower growing broilers are kept at a lower density and are also more frequently kept at farms with a low number of chicken houses compared to regular broilers, as shown in Figure D and E (6.5 supplementary figures). However, also genetic features may be involved, as chicken breeds have been shown to differ in their susceptibility to *Campylobacter* colonisation (Li, 2010; Stern, 1990). Several studies reported that *C. jejuni* affects broilers of regular and slower growing breeds differently. It was observed that while regular growing breeds had wet litter post infection and thus high level of pododermatitis, as well as severe diarrhoea, the slower growing breeds had dry litter and less pododermatitis and no diarrhoea (Williams, 2013). The breed, i.e. regular and slow growing was a variable added to the multivariate logistic regression model, however did not remain in the final one. The *Campylobacter* colonisation in broilers of two types of breed shall be further explored. The slower growing breeds became positive on average 12 days (CI 5; 18) later compared to the regular growing breeds. The reasons are not explained in the current study and further investigations of the differences between the animals and farm practices related to the breeds are needed.

Mortality is also reported as associated with *Campylobacter* presence in flocks (Bull, 2008). Although this variable came up in the univariate analysis, it did not remain in our final model.

Partial depopulation (thinning) is recognised as associated with *Campylobacter* presence in flocks (Hansson, 2010; Torralbo, 2014). With the current data set we have not found a statistically significant association between partial depopulation of flocks (thinning) and *Campylobacter* presence. This is in line with a study reported earlier in the Netherlands (Russa et al., 2005).
Lack of association in the current study might be explained by the limited number of observations available, since the figure on thinning in the Appendix 6.4 shows that the positive flocks were more often thinned. Not always the data on thinning was delivered. Usually this information was collected via VKI forms or forms used to deliver the samples. For several flocks however this was based on input from farmer and collected retrospectively. In addition thinning is generally recognised as a practice applied for regular growing broiler breeds. Therefore a presence of association between the thinning and *Campylobacter* status was analysed in the subset of available data on regular growing breeds, and it remained statistically insignificant. In the slower growing breeds the partial depopulation may occur as well (Table A. Appendix 6.5), however is not recognised as a thinning practice. Thus such practices shall be further observed and thinning definition adjusted for the slow growing breeds.

The variation between the farms was accounted for in the model by adding the farm as a random effect. The differences between farms and their management shall be further investigated. Variables as for e.g. stock density, light intensity and other influencing the performance of broilers could be taken into account in the further studies.

In the current study we measured the biosecurity practices on farms with the CAMPAS questionnaire in order to evaluate whether the results of the questionnaire correspond with the results on the *Campylobacter* presence on the farms. The goal is to have a questionnaire as a cost-effective tool for farmers to detect (strengths and) weaknesses in their biosecurity level which results in a higher chance of *Campylobacter* introduction on these farms. It was expected that higher CAMPAS scores (poorer biosecurity) would be observed for farms having more frequently positive flocks and lower scores for farms having more frequently negative flocks. This was not always the case. The final multivariate logistic regression model used to analyse associations between the CAMPAS outcome an *Campylobacter* results revealed four factors associated with presence of *Campylobacter* in broiler flocks. One of them was the higher number of houses on the farm what is in line with reports published earlier (Bouwknegt et al., 2004; McDowell, 2008). In addition the higher score on farm hygiene (higher score indicated poorer hygiene on farm) was associated with *Campylobacter* presence, what corresponds with findings of other authors (Hansson, 2010). However two other indicators remaining in the model as pest control score and farm premises score revealed association in an opposite direction than expected, i.e. the higher the score

(indicating poorer pest control practices and poorer biosecurity practices on the farm premises) the less positive the farm. These indicators require further revision, since for example other studies reported association between presence of *Campylobacter* and presence of rodents at farm (McDowell, 2008). Potentially the farms struggling more frequently with the rodents might have more measures implemented. In the CAMPAS questionnaire the questions were weighed similarly, however some factors might carry a higher risk than others. Potentially the scores in particular categories shall be weighted, questions rephrased, categories merged or confounders investigated. Further the farmers volunteered to participate in the study and to answer the CAMPAS questionnaire and this group may therefore not be true representatives of the Dutch broiler industry. Potentially all of the farms participating in the study may score rather high in the biosecurity procedures. Evaluation of the scores from the national biosecurity program (IKB Hygiënescan) seem to support this assumption.

Collection of the information with the help of log books occurred to be very challenging. Sometimes the farmers reported that no unusual activities happened on their facilities and thus returned an empty log book. Therefore potentially some activities were not recorded in the log books, since they were considered as usual. It would be worthwhile to collect such information with the help of cameras or other means to monitor compliance of farmers with biosecurity procedures in the real time. Such information could be summarised and analysed with the help of modern techniques for more reliable insight in daily farming practices. This approach would require a huge contribution from farmers and their willingness to participate in a study on such real time monitoring of their daily practices.

With respect to the investigation of the flies traps, no correlation between number of flies and status of selected flocks was found. In general the farmers placed the traps at different locations, thus this could be more standardised in future studies.

In conclusion seasonal effect confirmed to be the most prominent risk factor of *Campylobacter* presence in broiler flocks. Additional factors associated with increase in the *Campylobacter* presence in flocks included mowing of premises and the *Campylobacter* status of previous flock. These all factors point to the role of farm management practices. These are still not well defined and captured under the current study and require detailed tracking of actions taken on farm. Furthermore most of the biosecurity related information was gathered by questionnaire and practices reported by the farmers. These were not validated by observations. This may be considered as a limitation, since compliance to biosecurity protocols might not have been captured accurately. This points to the need to gain insight in the actual behaviour and compliance of farmers to biosecurity practices on farms.

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6 Appendix

6.1 CAMPAS Checklist Campylobacter op vleeskuikenbedrijf

Algemeen

Bedrijfsnaam	
Naam pluimveehouder	
Locatie	
Datum bedrijfsbezoek	
IKB Certificering	
Status Campylobacter	
Staltype	
Aantal vleeskuikens	
Neventak	

Bedrijfstype en kenmerken

Stal	Aantal dieren	Leeftijd	Soort pluimvee	Houderijsysteem
1				
2				
3				
4				

0. Omgeving

0.1	Heeft u op andere locaties ook nog pluimveebedrijven?	Ja/Nee	Indien ja, 1 punt	
0.2	Is er een andere pluimveehouder binnen een straal van 3 km van uw bedrijf?		Indien ja, 1 punt	
0.3	Bevindt uw bedrijf zich in een pluimveerijk gebied?		Indien ja, 1 punt	

1. Bedrijfsterrein

1.1	Is het bedrijfsterrein afgesloten met een hek of ketting?		Indien nee, 1 punt	
1.2	Is het terrein rondom de stallen vrij van materialen?		Indien nee, 1 punt	
1.3	Is het terrein schoon en opgeruimd?		Indien nee, 1 punt	
1.4	Is het terrein rondom de stallen vrij van begroeiing?		Indien nee, 1 punt	
1.5	Zijn de loop- en rijpaden rondom de stallen verhard?		Indien nee, 1 punt	
1.6	Zijn vleeskuikens de enige landbouwhuisdieren op de locatie?		Indien nee 1 punt	
1.7	Komen andere landbouwhuisdieren voor in de directe omgeving van de locatie?		Indien ja, 1 punt	
1.8	Wordt mest uitgereden van landbouwhuisdieren in de directe omgeving van de stallen?(weide/land)		Indien ja, 1 punt	

1.9	Zijn open mesthopen/opslag aanwezig op het bedrijfsterrein?		Indien ja, 1 punt	
1.10	Komen huisdieren (honden/katten/..) voor op het bedrijfsterrein?		Indien ja, 1 punt	
1.11	Komen huisdieren (honden/katten/..) voor in de pluimveestallen?		Indien ja, 1 punt	
1.12	Gaat de aan- en afvoer van voer e.d. volgens het schone en vuile weg principe?		Indien nee, 1 punt	
1.13	Wordt alle voeder (tevens ruwvoeders van bijvoorbeeld eigen teelt) opgeslagen in gesloten silo's waar vogels of ongedierte niet bij kunnen?		Indien nee, 1 punt	
1.14	Wordt afval bewaard in afgesloten containers?		Indien nee, 1 punt	
1.15	Wordt stro/strooisel en afleidingsmateriaal zo opgeslagen dat er geen vogels of ongedierte bij kunnen?		Indien nee, 1 punt	
1.16	Staan voersilo's op een verharde ondergrond?		Indien nee, 1 punt	
1.17	Worden eventuele voerresten direct verwijderd?		Indien nee, 1 punt	
1.18	Loopt afvoer van water van daken van bedrijfsgebouwen via dakgoten/regenbuizen?		Indien nee, 1 punt	
1.19	Is het bedrijfsterrein goed ontwaterd en vrij van vijvers en wateropvang?		Indien nee, 1 punt	

2. Bedrijfshygiëne

2.1	Wordt all-in- all-out op bedrijfsniveau toegepast?		Indien nee, 1 punt	
2.2	Is een hygiënesluis/omkleedruimte aanwezig op de scheiding van het schone en vuile bedrijfsgedeelte?		Indien nee, 1 punt	
2.3	Is een goed zichtbaar hygiëne-instructieprotocol aanwezig voor bezoekers?		Indien nee, 1 punt	
2.4	Is een voorziening voor het wassen van de handen aanwezig?		Indien nee, 1 punt	
2.5	Is een operationele douche aanwezig?		Indien nee, 1 punt	
2.6	Betreden medewerkers en bezoekers het 'schone' bedrijfsgedeelte altijd via een hygiënesluis?		Indien nee, 1 punt	
2.7	Maakt iedereen op het bedrijf gebruik van bedrijfseigen kleding/wegwerpkleding?		Indien nee, 1 punt	
2.8	Maakt iedereen op het bedrijf gebruik van bedrijfseigen schoeisel/overschoentjes?		Indien nee, 1 punt	
2.9	Wordt bedrijfs- en stalkleding na ieder gebruik gewassen?		Indien nee, 1 punt	
2.10	Wordt de erfverharding gereinigd na ontvangst van eendagskuikens?		Indien nee, 1 punt	

2.11	Wordt de erfverharding ontsmet na ontvangst van eendagskuikens?		Indien nee, 1 punt	
2.12*	Wordt de erfverharding gereinigd voor en na het uitladen?		Indien nee, 1 punt	
2.13*	Wordt de erfverharding ontsmet voor en na het uitladen?		Indien nee, 1 punt	

* Indien geen uitladen wordt toegepast, dan "niet van toepassing". Score wordt dan aangepast (deze vragen tellen dan niet mee).

3. Stalhygiëne

3.1	Zijn gaten of kieren in de buitenmuren of deuren(inclusief luchtinlaten) aanwezig waar ongedierte- of insecten door naar binnen kunnen komen?		Indien ja, 1 punt	
3.2	Zijn openingen van ramen of luchtinlaten aanwezig waar vogels door heen kunnen komen?		Indien ja, 1 punt	
3.3	Zijn drangers aanwezig op alle loopdeuren?		Indien nee, 1 punt	
3.4	Zijn de oppervlakten in de stal glad, zonder beschadigingen, gaten, kieren (en daardoor goed te reinigen?)		Indien nee, 1 punt	
3.5	Is een betonnen of asfalt verharding voor de toegangsdeuren aanwezig?		Indien nee, 1 punt	
3.6	Is een voorlokaal aanwezig, afgescheiden van de dierverblijven?		Indien nee, 1 punt	
3.7	Is er slechts één toegang aanwezig tot de vleeskuikenstal tijdens de productieperiode?		Indien nee, 1 punt	
3.8	Is een schoeiselontsmettingsbak of -mat aanwezig bij de entree?		Indien nee, 1 punt	
3.9	Is er een strikte, fysieke scheiding tussen het schone en vuile gedeelte?		Indien nee, 1 punt	
3.10	Zijn mondkapjes, hoofdbedekkingen en handschoenen aanwezig/gebruikt?		Indien nee, 1 punt	
3.11	Is er een voorziening aanwezig om handen te wassen met zeep?		Indien nee, 1 punt	
3.12	Wordt schoeisel altijd gewisseld voor het betreden van de dierruimten?		Indien nee, 1 punt	
3.13	Wordt altijd omgekleed in staleigen kleding en schoeisel voor de dierruimten worden betreed?		Indien nee, 1 punt	
3.14	Wordt staleigen kleding en schoeisel enkel gedragen in de dierruimten en het schone deel? (nooit mee naar buiten)		Indien nee, 1 punt	
3.15	Worden de handen voor het betreden van de dierruimten altijd gewassen en gedesinfecteerd?		Indien nee, 1 punt	
3.16	Zijn de afvalbakken afgesloten?		Indien nee, 1 punt	
3.17	Wordt het voorlokaal frequent bezemschoon gemaakt?		Indien nee, 1 punt	

3.18	Worden alle staloppervlakten gereinigd en ontsmet tussen de rondes?		Indien nee, 1 punt	
3.19	Wordt alle apparatuur (drinkers, voerpannen) grondig gereinigd en ontsmet tussen de rondes?		Indien nee, 1 punt	
3.20$	Zijn de materialen van de vangploeg gereinigd en ontsmet voordat de ploeg het bedrijf betreedt?		Indien nee, 1 punt	
3.21*	Neemt de vangploeg specifieke IKB hygiënemaatregelen in acht bij het uitladen?		Indien nee, 1 punt	

* Indien geen uitladen wordt toegepast, dan "niet van toepassing". Score wordt dan aangepast (deze vraag telt dan niet mee).

$ Indien bedrijfseigen materialen worden gebruikt, dan "niet van toepassing". Score wordt dan aangepast (deze vraag telt dan niet mee).

4. Materialen en voertuigen

4.1	Worden de wielen en wielkasten van alle wagens voor het betreden van het bedrijfsterrein gereinigd/ontsmet?		Indien nee, 1 punt	
4.2	Worden de wielen en wielkasten van alle wagens bij het verlaten van het bedrijfsterrein gereinigd/ontsmet?		Indien nee, 1 punt	
4.3	Wordt uitsluitend gebruik gemaakt van bedrijfseigen materialen (vaccinatieapparatuur, gereedschap etc.)		Indien nee, 1 punt	
4.4	Zijn alle in de stal benodigde materialen en -hulpmiddelen, zoals emmers etc. staleigen?		Indien nee, 1 punt	
4.5	Worden alle staleigen materialen en hulpmiddelen gereinigd en ontsmet tussen opeenvolgende rondes?		Indien nee, 1 punt	
4.6	Worden dode dieren dagelijks uit de stal verwijderd?		Indien nee, 1 punt	
4.7	Is de kadaveropslag gekoeld, afsluitbaar en visueel schoon?		Indien nee, 1 punt	
4.8	Worden hulpmiddelen voor het verplaatsen van dode dieren gereinigd en ontsmet na gebruik?		Indien nee, 1 punt	
4.9	Bevindt de aanbiedingsplaats van kadavers zich buiten of aan de buitenrand van het bedrijfsterrein?		Indien nee, 1 punt	
4.10	Worden kadaverbakken/tonnen altijd gereinigd en ontsmet na het legen?		Indien nee, 1 punt	
4.11	Wordt mest direct na het leegkomen van de stal verwijderd?		Indien nee, 1 punt	
4.12	Wordt mest afgevoerd in een afgedekte/gesloten mestcontainer/mesttrailer?		Indien nee, 1 punt	
4.13	Zijn mestcontainers/mesttrailers visueel schoon voordat deze op het bedrijfsterrein worden toegelaten?		Indien nee, 1 punt	
4.14	Is mest van elke productieronde volledig afgevoerd van het bedrijfsterrein direct na het afleveren van de dieren?		Indien nee, 1 punt	

4.15	Wordt een eventuele mestplaats na afvoer van de mest gereinigd en ontsmet?		Indien nee, 1 punt	
4.16	Wordt erfverharding na afvoer van mest gereinigd?		Indien nee, 1 punt	
4.17	Wordt erfverharding na afvoer van mest ontsmet?		Indien nee, 1 punt	

5. Ongediertewering- en bestrijding

5.1	Wordt ongediertebestrijding uitgevoerd door een professioneel bedrijf dat hiervoor erkenning heeft (zoals IKB-PBS erkenning)		Indien nee, 1 punt	
5.2	Is er een ongediertebestrijdingsplan voor het weren en bestrijden van ratten en muizen rond de pluimveestallen?		Indien nee, 1 punt	
5.3	Vind wisseling van werkzame stof in ongediertebestrijdingsmiddelen frequent plaats?		Indien nee, 1 punt	
5.4	Wordt het aanwezige grasland rondom de stallen kort gehouden?		Indien nee, 1 punt	
5.5	Zijn de stallen vrij van ratten en muizen (of uitwerpselen en vraat)?		Indien nee, 1 punt	
5.6	Zijn de stallen vrij van wilde vogels?		Indien nee, 1 punt	
5.7	Zijn de stallen vrij van insecten?		Indien nee, 1 punt	
5.8	Kan vogelpoep binnen komen via luchtin- of uitlaten (met name via het dak)?		Indien ja, 1 punt	
5.9	Vindt vliegenbestrijding plaats in het voorlokaal?		Indien nee, 1 punt	
5.10	Vindt vliegenbestrijding plaats in de stal?		Indien nee, 1 punt	
5.11	Wordt het binnenkomen van insecten tegen gegaan (door bijvoorbeeld een luchtgordijn)?		Indien nee, 1 punt	
5.12	Wordt specifiek aandacht besteed aan het bestrijden van insecten/eieren in gaten en kieren van vloeren en wanden?		Indien ja, 1 punt	
5.13	Is het bedrijf vrij van hobby pluimvee?		Indien nee, 1 punt	

Indien een vraag op het bedrijf niet van toepassing is, dient deze te worden overgeslagen. Het is hierbij van belang dat de formule wordt aangepast, zodat de berekening in het Campas blijft kloppen.

6.2 Log book

Indication of activities monitored by the log book.

Gebeurtenissen	Datum	Datum	Datum	Datum	Datum	Datum	Datum
Buiten							
Mestopslag vorige ronde in nabijheid van stal(len)							
Grasmaalen rondon stal(len)							
Uitrijden van mest op percelen naast stal(len)							
Ploegen van percelen naast stal(len)							
Maalen van percelen naast stal(len)							
Spuiten van percelen naast stallen							
Afvoer mest door transporteur							
Aanvegen erfverharding							
Onderhoud/reparatie buitenkant stal(len)							
Ongedierte bestrijdling on de stal(len)							
Anders:							
Voorlokaal							
Met buitenschoeisel voorlokaal in							
Deur voorlokaal heeft tijd open gestaan							
Reparatie door externe(n) in voorlokaal							
Tussentijds inbrengen van voerzakken/strooisel in voorlokaal							
Huisdier (hond, kat) in voorlokaal							
Bezoeker zonder bedrijfseigen schoeisel/overkleding in voorlokaal							
Ongedierte in voorlokaal gezien (ratten, muizen)							
Anders							
Dierverblijf							
Met buitenschoeisel dierverblijf in							
Bezoeker zonder bedrijfseigen schoeisel/overkleding in dierverblijf							
Naam bezoker+opdracht/taak (DA vaccinatie/monitorimg/visitie, enz.)(voorlichter)							
Bij uitladen: onvoldoende hygiene vangploeg							
Reparatie door externe(n) in stal							
Tussentijds inbrengen van stro/strooisel in stal							
Ventilatie-alarm							
Ongedierte in dierverblijf gezien (ratten, muizen)							
Ongediertebestrijding							
Anders							

6.3 Protocol for collection of faecal samples and submission form

Uw kenmerk van inzending: PPS Campylobacter
Projectnummer 4400001156

Eigenaar:		Diersoort:	Pluimvee
		Materiaal	Faeces
Naam eigenaar		Onderzoek	Campylobacter PCR (CAM04)
Adres		Aantal monsters	
Woonplaats		Datum inzending	
e-mailadres (voor versturen van uitslag*)			

Deze monsters bewaren/zijn bewaard in de vriezer

Protocol voor het nemen van faecesmonster voor *Campylobacter*onderzoek bij pluimvee (PPS 'beheersing van *Campylobacter* in pluimveeketen')

1. Per te onderzoeken stal worden **4-6 hoopjes mest** genomen (hoeft geen caecale mest te zijn, maar wel vers!). Hoopjes mest van één stal kunnen samen in een monsterpotje worden gedaan.
2. De mestmonsters worden van **verschillende plekken in de stal** genomen om de pakkans te vergroten.
3. Monsterpotje niet helemaal tot bovenaan toe vullen (in verband met mogelijke gasvorming en daarmee oplopende druk in potje) en goed sluiten.
4. **Gegevens invullen**: grijs gearceerde velden hierboven en op de achterkant.
5. Na monstername worden potje(s) met formulieren voor onderzoek aangeleverd bij: Wageningen Bioveterinary Research
   Afdeling DSU
   Houtribweg 39
   8221 RA Lelystad
   Contactpersoon laboratoriumonderzoek: Miriam Koene. Tel 0320 - 238 425

1. **Wanneer zijn de dieren geplaatst?**

stal 1							
d	d	m	m	j	j	j	j

stal 2							
d	d	m	m	j	j	j	j

2. **Wat zijn de data waarop de monsters zijn genomen?**

stal 1							
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j

stal 2							
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j
d	d	m	m	j	j	j	j

3. **Wanneer zijn de bemonsterde dieren geslacht?**
   (indien er uitgeladen wordt, zowel datum van uitladen als wegladen)

uitladen

d	d	m	m	j	j	j	j

uitladen

d	d	m	m	j	j	j	j

wegladen

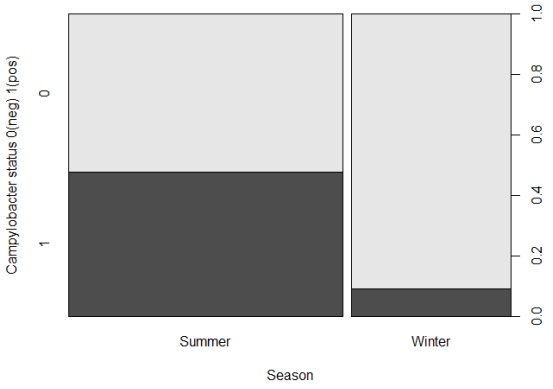
d	d	m	m	j	j	j	j

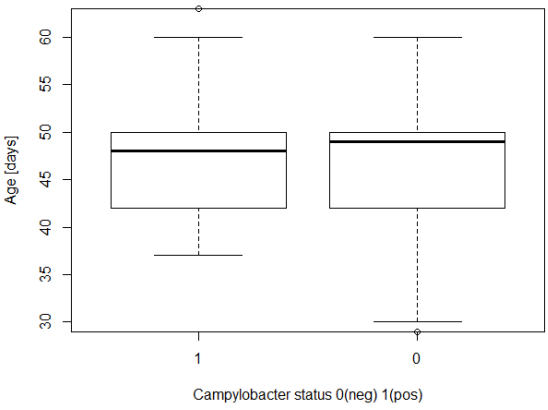
wegladen

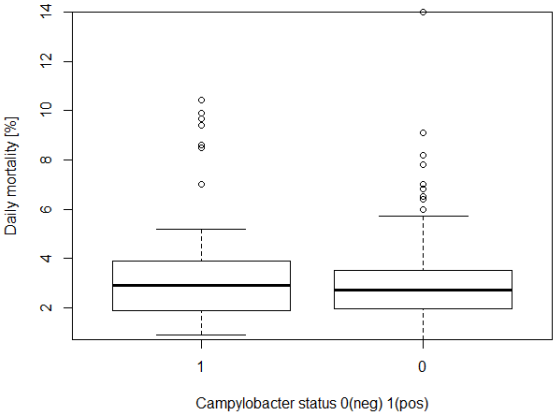
d	d	m	m	j	j	j	j

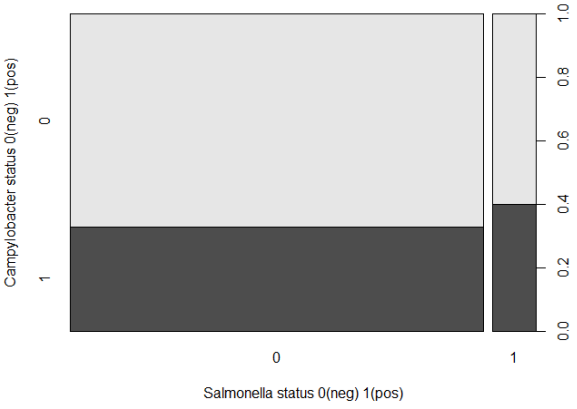
6.4 Explanatory variables versus *Campylobacter* status

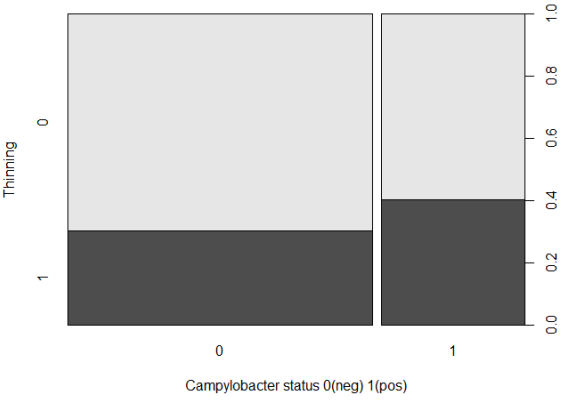
Plots summarising explanatory variables on flock level versus *Campylobacter* status of the flocks. Data base included 209 flocks (after eliminating the flocks with missing variables).

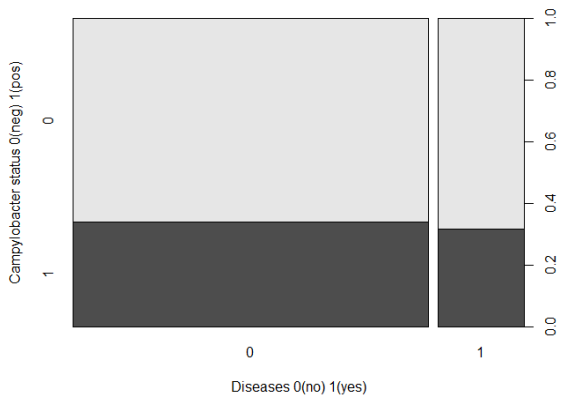


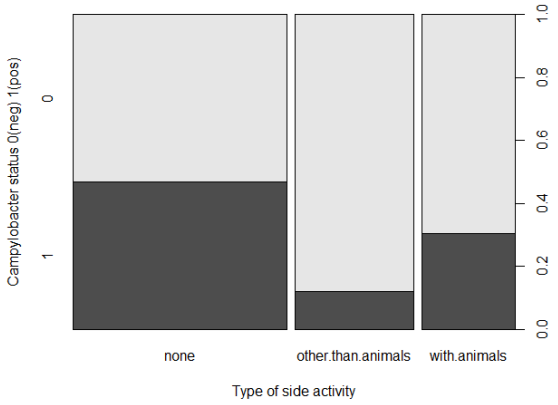


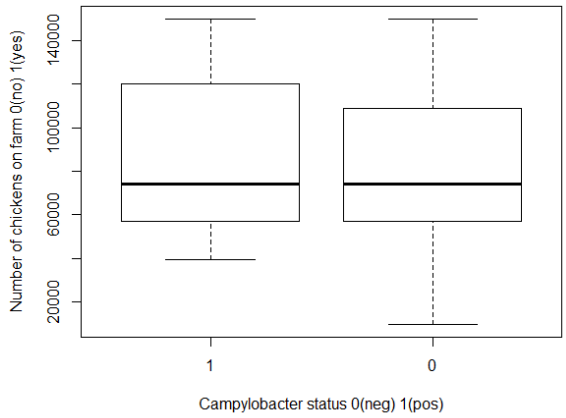


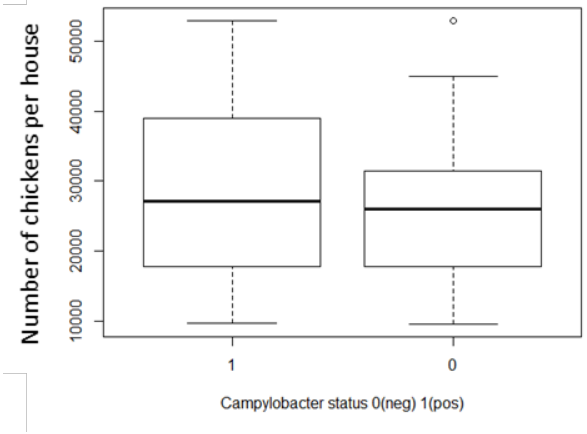


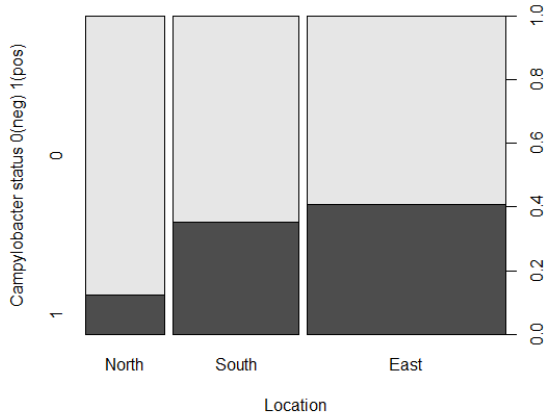


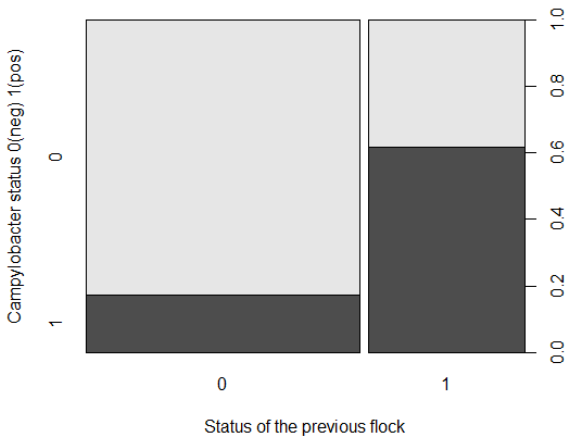

Campylobacter status 0(neg) 1(pos)
0
1
0.0
0.2
0.4
0.6
0.8
1.0
0
1
Diseases 0(no) 1(yes)

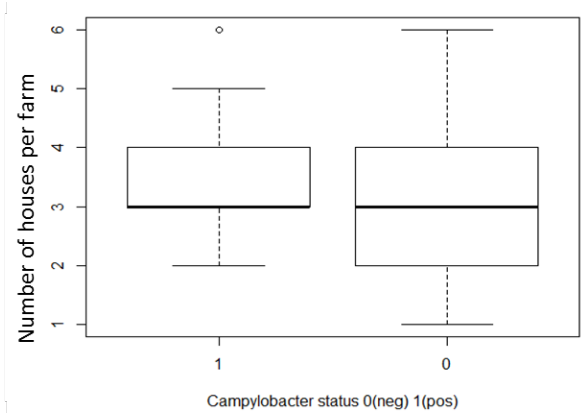

Campylobacter status 0(neg) 1(pos)
0
1
0.0
0.2
0.4
0.6
0.8
1.0
none
other.than.animals
with.animals
Type of side activity


Number of chickens on farm 0(no) 1(yes)
20000
60000
100000
140000
1
0
Campylobacter status 0(neg) 1(pos)


Number of chickens per house
10000
20000
30000
40000
50000
1
0
Campylobacter status 0(neg) 1(pos)


Campylobacter status 0(neg) 1(pos)
0
1
0.0
0.2
0.4
0.6
0.8
1.0
North
South
East
Location


Campylobacter status 0(neg) 1(pos)
0
1
0.0
0.2
0.4
0.6
0.8
1.0
0
1
Status of the previous flock


Number of houses per farm
1
2
3
4
5
6
1
0
Campylobacter status 0(neg) 1(pos)

6.5 Supplementary figures

Figure A. Percentage of *Campylobacter* positive flocks per months (separated period 2017 and 2018).

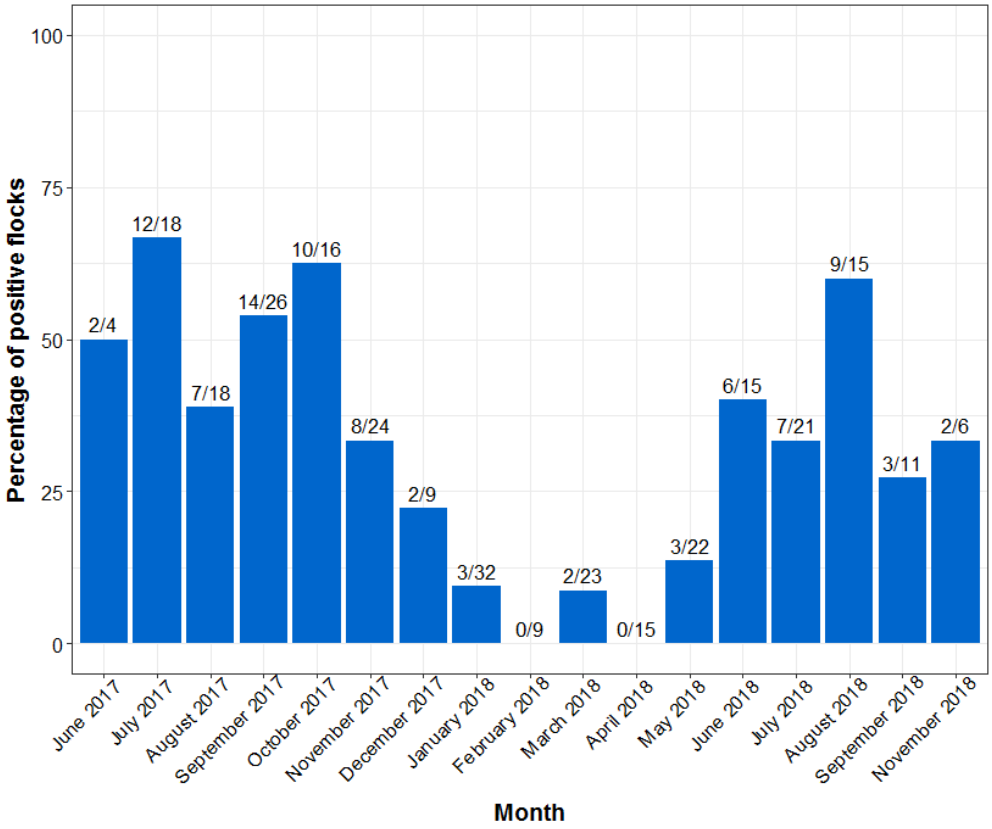


Figure B. Number of positive (blue) and negative (green) flocks per farm.

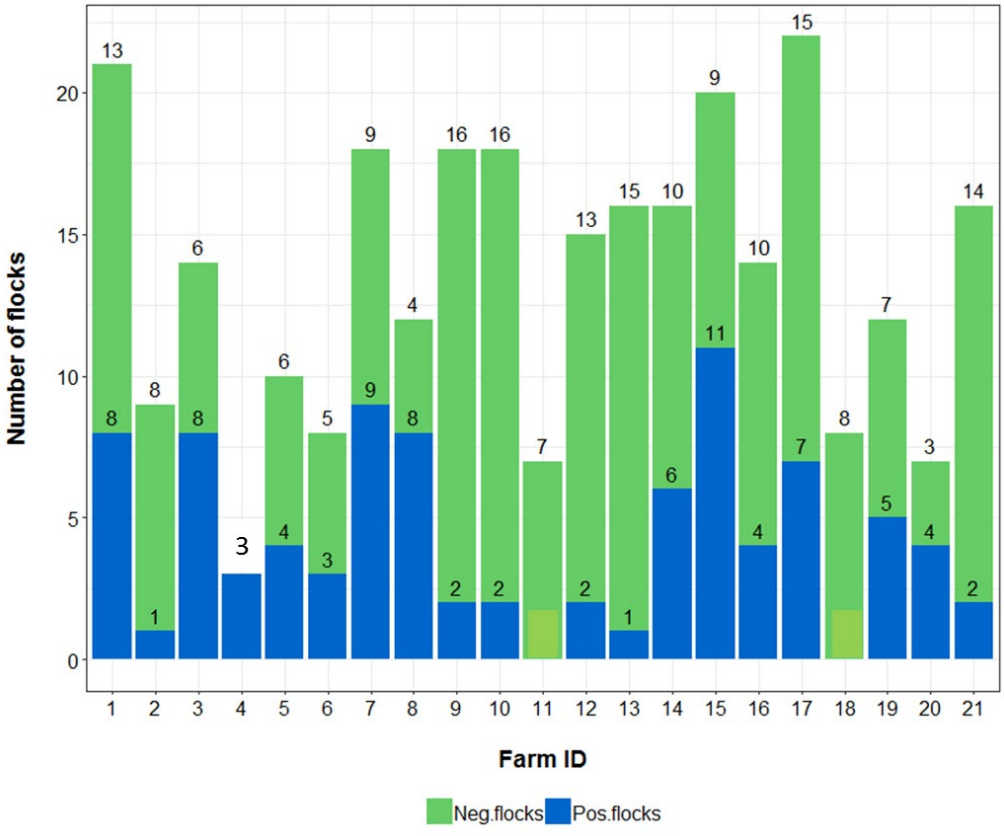


Figure C. Side activity performed on farm versus number of broilers on the farm.

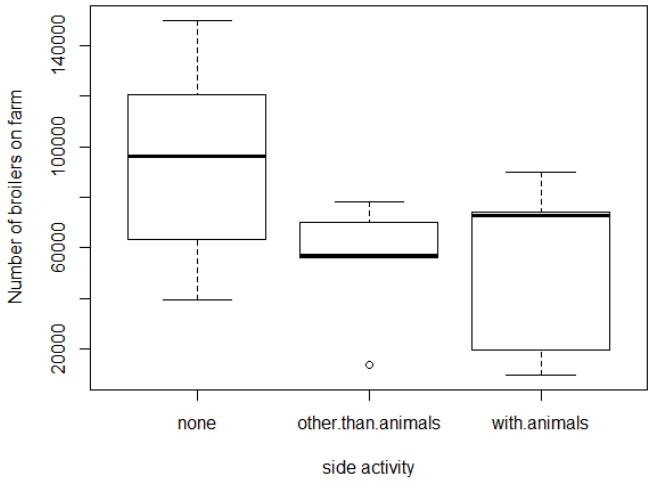


Figure D. Breed versus number of broilers on the farm.

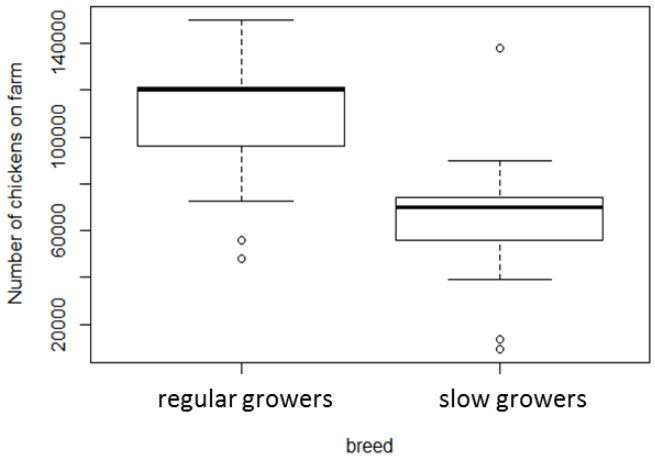


Figure E. Breed versus number of houses on the farm.

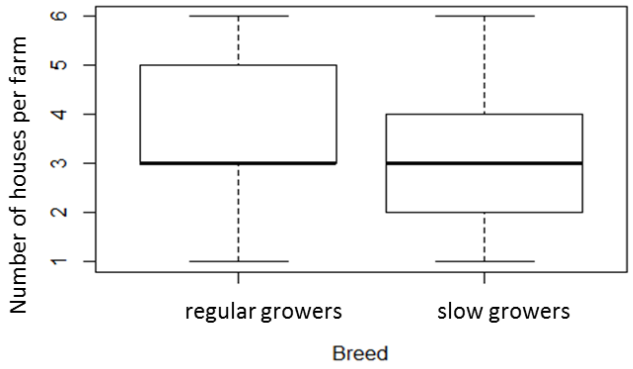


Figure F. Slaughter age versus breed

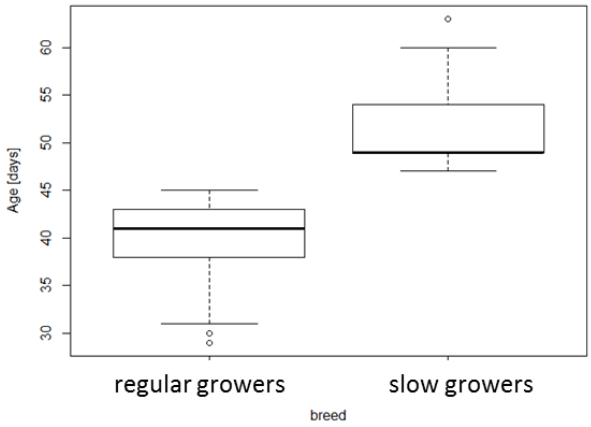


Figure G. Breed versus mortality

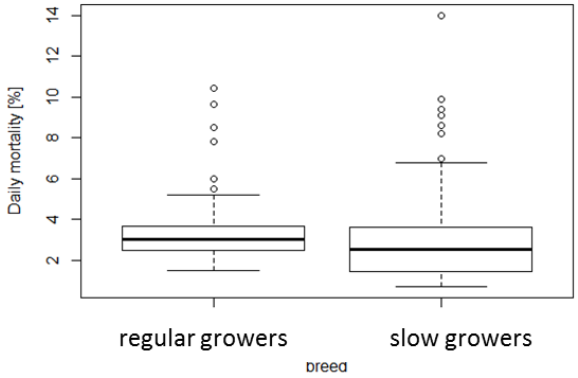


Figure H. Number of houses per farm versus side activity

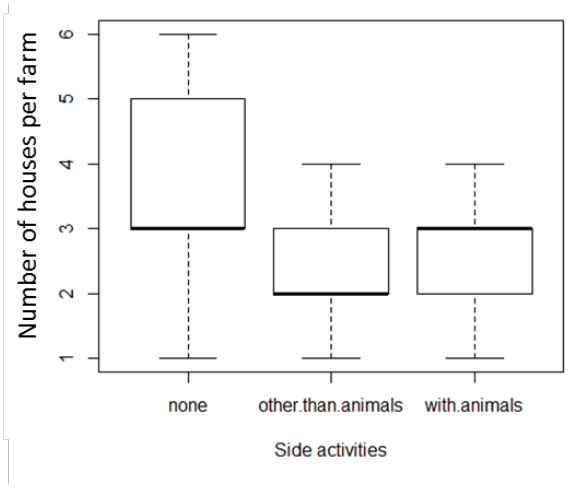


Table A. Results of the Chi-squared test between categories of breed and thinning (0 no, 1 yes)

	Thinning	
Breed	Not done (0)	Done (1)
regular growers	13	77
slow growers	157	3

Pearson's Chi-squared test p-value < 0.01