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Salmonella in Belgian laying hens: an Identification of Risk Factors

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Abstract

Since the 1980s, the prevalence of *Salmonella* in Belgian poultry layers and broilers has greatly fluctuated with a rise observed in 2003 and a significant decrease in 2005. In order to alleviate the risk at egg consumer level, it is crucial to understand the factors which influence the contamination and the spread of Salmonella in laying hens. To study such determinants we explored the Belgian data from the 2005 baseline study on the prevalence of *Salmonella* in laying flocks of Gallus gallus in the European Union. The response variables corresponded to presence or absence of *Salmonella* from dust and faecal samples taken from the environment of a Belgian layer flock. The explanatory variables included: region of Belgium, sampling time (month the flock was sampled), production type (cage or barn/ free range), *Salmonella* vaccination status, flock age and flock size. Analyses of these data were performed using a bivariate logistic regression model assuming independence between the

two responses and bivariate Generalized Estimating Equations model, which incorporates the correlation between the two responses on the same flock. The main risk factor that was identified was rearing flocks in cages compared to barns and free-range systems. The results also showed a significant higher risk for *Salmonella* for a one week increase in flocks' age as well as with a unit increase in the size of the flock.

Key words: *Salmonella*; laying hens; production type; within-flock correlation.

Introduction

Salmonellosis constitutes a major public health burden and represents a significant cost in many countries. In Belgium, the disease ranks high among the reported food borne illnesses (Collard *et al*., 2004). Even if the incidence of human salmonellosis has diminished since 1999, in 2004, 9545 cases were reported in the country (EFSA, 2006a). As in most of the countries around the world, Belgian *Salmonella* outbreaks in humans are very often linked to the consumption of contaminated eggs (Davies and Breslin, 2001a; Van Immerseel *et al.*, 2005). The most frequently isolated serotype in layer flocks in the EU as well as in Belgium is *Salmonella* Enteritidis which is a non-typhoid non-host adapted serotype with a very wide host range (Baird-Parker, 1990; Gast *et al*, 2005; Quinet, 2005; VAR, 2005; EFSA, 2004). The bacterium infects the eggs by two processes: first by vertical transmission during the development of the egg within the ovary or its passage through the oviduct and secondly by horizontal transmission through trans-shell contamination (Kinde *et al*., 2000; WHO FAO, 2002; Davies and Breslin, 2003a; Van Immerseel *et al*., 2005). Vertical transmission is considered to be the major route of egg contamination and should be controlled by applying sanitary measures at the breeders level (that is, hygiene practices and eventually vaccination) while horizontal transmission should be reduced by preventing contacts between the layer

hens and by cleaning and disinfecting the flock's environment. *Salmonella* is known for its ability to asymptomatically infect the hen's oviduct (De Buck *et al*., 2004a; 2004b). Therefore detection of infected flocks depends entirely on laboratory analysis. An infected hen may contaminate one egg out of 200 (Quinet, 2005). Reducing *Salmonella* flock prevalence results in a directly proportional reduction in human health risk (Altekruse *et al*., 1993). This suggests that sanitary measures at the flock level contribute to a significant reduction of the risk for salmonellosis due to egg consumption. In Belgium, the layer breeders are not significantly infected, probably due to the many years' efforts of control at this level and therefore, it is reasonable to assume that most day-old chicks are free from *Salmonella* when placed on farms (Davies and Breslin, 2001; AFSCA, 2004). The majority of the infections in layer hens seem to be attributed to the persistent contamination of the farm. Indeed, the presence of Salmonella in the laying house environment has been strongly correlated with the probability that hens will lay contaminated eggs. Chicken are infected after oral ingestion of the bacteria from the environmental sources (for example, contaminated fluff, dust, feed etc) invasion of the mucosal epithelial cells, which leads to systemic dissemination and colonization of the ovary and oviduct (Henzler *et al*., 1998; Davies and Breslin, 2003b). The primary control should focus at farm level. Control measures include preventing contacts with contaminated feed and visitors, wearing house-specific clothing, thorough cleaning and disinfection of the layer houses, vaccination, rodent control programs. In Belgium, every holding housing more than 5000 hens is required to be sampled for *Salmonella* diagnosis 3 weeks before slaughter time. This measure probably has contributed to a reduction of the risk for food-borne salmonellosis. However, in 2004, still 27% of the layer flocks analysed remained positive for *Salmonella* (AFSCA, 2004). Several risk factors have been described, but in order to advise the Belgian competent authority (Federal Agency for the safety of the Food Chain) with detailed, practical guidance, an

understanding of possible causal factors is essential. The objective of the study reported here is to investigate the risk factors which are associated with the occurrence of *Salmonella* in laying hens in Belgium using data collected for the Baseline Study on the Prevalence of *Salmonella* in laying flocks of *Gallus gallus* f. domestica in the European Union (SANCO/34/2004 and Commission decision 2004/665/EG). Although it would be worthwhile to utilize data from earlier years, the 2005 data set contained flock information, particularly on some demographic factors and *Salmonella* vaccination status, which were unavailable for earlier databases.

Materials and methods

Data collection

The Belgian part of the Baseline Study on the Prevalence of *Salmonella* in egg laying flocks of Gallus gallus in the European Union consisted of a cross-sectional study that covered the year 2005 from February to September in Belgium. The primary sample size providing the number of holdings which had to be tested was calculated on the basis of a target prevalence of 20%, a confidence level of 95% and an accuracy of 3% (Commission decision 2004/665/EG). The population of laying hens was stratified according to holding size (below 1000, 1000-2999, 3000-4999, 5000-9999, 10000-29999, 30000 and more). The number of holdings to be sampled was subsequently distributed proportionally to the number of holdings in each class. In all cases, only one flock per holding was sampled. Seven different samples, two dust samples and five faecal samples were collected from each selected flock. The dust samples were any of these types: 1) dust from different places in case of barn or free range flocks, 2) dust from egg belts, 3) dusty material from beneath cages. Faecal samples were any of these types: 1) boot swabs which are indeed socks placed over the boots. They are sufficiently absorptive to collect faecal or moist litter samples from the floor

surfaces (SANCO/34/2004 and Commission decision 2004/665/EG). 2) Pooled faecal samples from dip pits, 3) pooled faecal samples from dropping belts, 4) pooled faecal samples from scrapers. The collection of these samples was as follows: There had to be five pooled faecal samples taken per selected flock. For the pooled faecal samples in cages, there are normally several stacks of cages within a henhouse. The material from each stack picked up using a new pair of plastic gloves for each individual sample was included in each of the five pooled faecal samples of 200-300 grams. For the boot swabs in barns and free range flocks, each henhouse was divided in sectors of at least 100m that were walked on with new boot swabs, five pairs of boot swabs per henhouse. Each of the five pooled samples comprised of faecal material fixed to a pair of boot swabs. The dust material from beneath cages was obtained from 20 separate locations within a henhouse using a new pair of plastic gloves for each sample. Finally for the dust from different places from barns and free range, each dust sample was collected in a 250ml plastic jar or bag ensuring that all parts of the henhouse like from exhaust fan, ledges, beams etcetera were covered. In order to maximise sensitivity both faecal material (5 out of 7) and dust material from the environment (2 out of 7) were sampled, depending on whether the birds were reared in cages or barns or free-range, in such a way that the complete farm was represented. The hens were sampled at the end of their laying period, within a maximum of 9 weeks before depopulation. Samples were sent within 24 hours to the laboratory. The detection method was as recommended by the Community Reference Laboratory for Salmonella in Bilthoven, The Netherlands, that is, a modification of ISO 6579:2002. *Salmonella* isolates were serotyped following the Kaufmann-White scheme (Popoff, 2001; VAR, 2005).

Data description

Although the proportion of flocks infected with *Salmonella* may significantly differ depending on the type of sample that was used (Kinde *et al.*, 2005), for the analyses in this study we grouped the three dust-type samples to form the 'dust material' and the four faecal-type samples formed the 'faecal material' thus reducing the seven sample types to two for all flocks. The 'dust material' and 'faecal material' were considered as positive (outcome=1) when at least one of the dust or faecal samples respectively was positive. They were considered as negative (outcome=0) when all of the dust or faecal samples respectively were negative. The frequencies of infected flocks were obtained based on the 'dust material' and 'faecal material' separately. Since the two outcomes, one from the 'dust material' and one from the 'faecal material', occurred on each flock, it was important to examine if an association existed between them. This was done using the Pearson Chi-square test of independence (FREQUENCY procedure in SAS). Also the measure of this association was explored using the Pearson correlation coefficient with the SAS CORRELATION procedure. The existence of an association signalled the necessity for the two outcomes to be modelled jointly.

The explanatory variables used include: region (1= Walloon or 0= Flanders), sampling time (month the flock was sampled: February to September), production type (cage or barn/ free range), age (in weeks), flock size (number of hens in the flock considered) and vaccination status against *Salmonella* (yes, unknown, or no). The flocks were vaccinated against *Salmonella enterica,* serovar Enteritidis during the rearing period (one day to 18-20 weeks) with either a live or inactivated vaccine type although for some flocks the vaccine type was not known. The last dose was administered a few weeks before the onset of laying eggs. The pullets were kept in separated installations on the laying farm considering special conditions like temperature and light among others. The associations between presence and absence of

Salmonella and each of the categorical variables was investigated using the Pearson chi-Square test of independence (FREQUENCY procedure in SAS). To explore the relation of the outcomes with the continuous explanatory variables we used the mean.

Data analysis

In this study we modelled the probability of infection of a flock. Therefore, we carried out analyses for the dichotomized bivariate response where a flock was infected if at least one of the samples of the 'dust material' or 'faecal material' tested positive otherwise the flock was considered not infected. Since the 'dust material' and 'faecal material' responses were binary outcomes, a natural assumption for their distribution was the binomial distribution. Various approaches and models were used to model these data. The first approach to analyse these data was to perform separate analyses for the two outcomes, for example, by fitting a logistic model, $\text{logit}\left(P(Y_D = 1)\right) = \boldsymbol{X}^T \boldsymbol{\beta_D}$ for the dust outcome variable and another logistic model, $\text{logit}\left(P(Y_F = 1)\right) = \boldsymbol{X}^T \boldsymbol{\beta_F}$ for the faecal outcome variable. The probabilities of the presence of Salmonella, $P(Y_D = 1)$ in dust and $P(Y_F = 1)$ in faeces, were predicted as functions of explanatory variables contained in the $\boldsymbol{X}$ design matrix using the logit link function. The estimates of the model parameters, $\boldsymbol{\beta_D}$ and $\boldsymbol{\beta_F}$, were obtained using maximum likelihood estimation. This however would ignore the correlation between the two outcomes. Moreover the Pearson correlation coefficient showed a tendency for the two outcomes to relate positively, meaning that when the dust outcome was positive the faecal outcome tended to be positive as well or vice versa.

In the second approach we modelled both outcomes jointly as (Y_D, Y_F), for example; by fitting the Generalized Estimating Equations (GEE) model, introduced by Liang and Zeger (1986). In order to use maximum likelihood estimation the joint

probabilities: $P(Y_D = 1, Y_F = 1)$, $P(Y_D = 1, Y_F = 0)$, $P(Y_D = 0, Y_F = 1)$, and $P(Y_D = 0, Y_F = 0)$ must be assumed at each combination of explanatory variables. However, when there are many explanatory variables this is not practical especially if some are continuous (Agresti, 2002). An alternative to maximum likelihood fitting uses the quasi-likelihood. Instead of assuming a bivariate binomial distribution for (Y_D, Y_F), the quasi-likelihood method specifies a model for the means of the marginal distributions of Y_D and Y_F; a variance function describing how the variance of Y_D and Y_F depend on their means; and a pairwise correlation, $corr(Y_D, Y_F) = \rho$ between the outcomes. Therefore the model was applied to two sets of marginal binomial parameters $\{P(Y_D = 1)\}$ and $\{P(Y_F = 1)\}$. The marginal logit model is then of the form

$$\begin{bmatrix} \log it\{P(Y_D = 1)\} \\ \log it\{P(Y_F = 1)\} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}^T \boldsymbol{\beta_D} \\ \boldsymbol{X}^T \boldsymbol{\beta_F} \end{bmatrix}$$

The estimates to the model parameters were obtained as solutions of quasi-likelihood equations called generalized estimating equations. In this study we assumed an exchangeable working correlation structure. Essentially the correlation between the outcomes was estimated and then used to re-estimate the regression parameters and adjust the standard errors. An advantage of the GEE model is that the estimates are valid even if one misspecifies the variance-covariance structure (Agresti, 2002; Molenberghs and Verbeke, 2005). In addition the GEE model estimates the magnitude of the correlation between the outcomes taking into account the explanatory variables.

The models were fitted using the SAS GENMOD procedure. A parsimonious model was built based on the ordinary logistic model where the probability of *Salmonella* positivity was modelled by including one explanatory variable (two continuous and four categorical) at a time and the variables that had a p-value less than 0.25 were introduced in the multiple

logistic regression models. A stepwise automatic selection procedure was also used to supplement the model selection. The two criteria led to the same model. Along with the selected main factors, their two-way interactions were added to the model. Higher interactions were not considered in order to keep a reasonable number of parameters in regard to estimation. However, two-way interactions between categorical variables, for instance, production type by vaccination status resulted into observations with only one type of the outcomes causing difficulties in estimation. The interactions between categorical and continuous variables posed no estimation problems but were found to be non-significant. Therefore the final model considered eliminated the 'region' variable and the interactions. By fitting the final model the results for the risk factors were expressed as odds ratios along with their corresponding 95% confidence intervals and probability values. A probability value of less than 0.05 indicated a statistically significant result.

Results

Data description

In total, data was recorded for 148 flocks. In Figures 1 and 2, we show the number of flocks with positive or negative results for *Salmonella* for dust and faecal samples. The numbers at the top of the bars indicate the number of flocks in each category on the horizontal axis. Specific to the dust sample type, Figure 1 also shows that in 102 flocks no dust sample was *Salmonella* positive whereas 22 flocks had one positive dust sample and 24 flocks had both dust samples positive. A similar interpretation follows for the faecal sample type. Grouping the results from Figure 1 into *Salmonella* positive flocks (if at least one sample was *Salmonella* positive) and *Salmonella* negative flocks (if all samples were *Salmonella* negative) produces Figure 2. Considering the dust sample type, for instance, 102 out of 148 flocks were *Salmonella* negative while the 46 were positive for *Salmonella.* The frequencies

for the faecal sample type are interpreted in a similar manner. The Pearson chi-square statistic for the association between the two outcome variables was estimated at 66.60 ($p < 0.001$) which rejects the null hypothesis of no association between the dust and faecal outcomes. The Pearson correlation coefficient between the two outcomes was obtained as 0.6708 giving an indication of moderate to strong positive association.

Figure 1 here

Figure 2 here

Table 1 shows the distribution of the number of *Salmonella* positive and negative flocks per each categorical explanatory variable. The percentages of all flocks that were positive or negative and the associations between presence and absence of *Salmonella* and each of the categorical variables using Pearson chi-Square test of independence are also shown. For both sample types there seems to be significant (p-values <0.05) associations of production type and *Salmonella* vaccination status on the presence and absence of *Salmonella.*

Table 1 here

For the Salmonella positive group, the flocks' mean age (in weeks) was 74.87 and 76.15 while the mean flock size was 21929.22 and 22156.6 for dust and faecal materials respectively. Similarly, for the *Salmonella* negative group, the mean age was 70.75 and 70.11 while the mean flock size was 13912.28 and 13727.1 for dust and faecal materials respectively. The mean age and mean flock size were higher for the Salmonella infected flocks than for the uninfected ones, suggesting an increased risk for Salmonella as the hens get older and as the flock size increases.

Data analysis

Knowing that the two outcomes of *Salmonella* were from the same flock meant that analyses which take into account the dependence between the responses from the dust material and faecal material outcomes were more appropriate. However, in order to explore the changes in effects with the complexity of a model, we analysed the two outcomes separately, first with univariate simple logistic models (USLM) shown in column 2 of Table 2 and secondly using univariate multiple logistic models (UMLM) presented in column 3 of Table 2. In these separate analyses the dust and faecal datasets were assumed to be independent. These findings were then compared with the findings from the appropriate model, the bivariate multiple logistic model (BMLM) in column 4 of Table 2. Column 2 shows the odds ratios estimated from the univariate simple logistic model analyses where one covariate was entered in the model while column 3 gives the estimated odds ratios from the univariate multiple logistic regression model where other covariates were controlled for. The USLMs identified that rearing flocks in cages compared to barns and free-range, not vaccinating flocks, a unit increase of flock size and a one week increase in flock age as significant risk factors for *Salmonella* in Belgian layer flocks. Controlling for other factors in the UMLMs showed that rearing layer flocks in cages is a significant risk factor in both dust and faecal data sets whereas a one week increase in flock age and a unit increase of flock size were significant risk factors for the faecal dataset alone.

However in column 4, which presents the appropriate analysis for the data, the joint analysis of the two datasets with the correlation between dust and faecal outcomes modelled as an exchangeable working correlation using GEE we observed that, controlling for other variables, rearing layer flocks in cages was still a significant risk factor but flock age, flock size and the month of July became borderline significant while *Salmonella* vaccination status

turned non-significant. A working correlation of 0.7384 was estimated which indicates a strong positive association between the responses that was ignored by USLMs and UMLMs, which modelled the two responses separately. Therefore the bivariate GEE confirmed the strong association between the outcomes and estimated this association even higher compared to the exploratory measure, the Pearson correlation coefficient of 0.6708 that did not account for other factors. About the risk factors for *Salmonella* found in this study, the exploratory data analysis, which gave an indication of risk factors using Pearson chi-square test of independence for categorical variables (Table 1) and using means for the continuous variables, and the confirmatory analysis via modelling (Table 2) led to similar conclusions.

Table 2 here

Discussion

The prevalence of *Salmonella* in commercial holdings of laying hens in Belgium is relatively high, especially when compared to the northern European countries (EFSA, 2006b). However, it should be mentioned that Belgium has many laying hens compared to neighbouring countries (Quinet, 2005). The European survey was based on environmental sampling which is considered to be an accurate and representative indicator for the presence of *Salmonella* in layer flocks and for the probability that hens would lay contaminated eggs (Henzler *et al*., 1994; Kinde *et al*., 2005). The persistence of the pathogen in the intestinal tract is more important when infection occurs in young chicks, since bacterial clearance occurs more efficiently in adults. Genetically distinct lines of hens and various breeds can also be responsible for differences in the presence of *Salmonella* in the faeces of a contaminated animal. It is important to take these factors into account as the duration of this shedding can influence the detection of *Salmonella* in the threatening flocks (Kinde *et al*., 2000; Gast *et al*., 2005). Environmental sampling is not entirely reliable as it can miss flocks

which passed the peak of infection but which are still producing contaminated eggs (Kinde *et al.*, 1996; Davies and Breslin, 2004; Van Immerseel *et al.*, 2005). The fact that one specific type of sample would be more contaminated than others helped identify risk factors, for example, a high level of the bacteria in dust (two dust samples positive instead of one) could point out a problem due to the ventilation system in the hen house or may be associated with cleaning and disinfection of the house, or with insufficient rodent control. A study from Gast *et al.*, 1998 suggested that infection could, among other things, occur by oral ingestion of external surfaces contaminated by airborne movement of *Salmonella* during the feeding or pecking. From our findings, we saw differences in the statistical relations between the response variable and the predictors. For instance, the age factor was statistically associated to *Salmonella* status in the faecal dataset (p=0.05), while not significantly associated (p=0.439) in the dust dataset. The risk for *Salmonella* in cages versus barn and free range was twice as high in the dust dataset as in the faecal dataset (OR= 20.11 versus 10.27).

The major risk factor identified from the analysis was rearing flocks in cages compared to rearing in barns and free-range systems. The risk of contamination with *Salmonella* is thought to be higher when eggs are produced in non-cage systems, because of the greater exposure of layers to environmental contamination (Kinde *et al.*, 1996; EFSA, 2004). However, in practice, control is not easier in cage layer houses; due to the difficulty to efficiently disinfect the cages and the higher densities of birds which produce a larger volume of contaminated faeces and dust (Davies and Breslin, 2004). The result of the current study clearly corroborates this finding. In addition, a clear difference was noticed in the proportions of vaccinated hens in the two types of production systems: 88% of the barn and free-range birds were vaccinated, while only 53% for the cage system poultry. The vaccination variable can act here as a confounding factor on the apparent association between production type and *Salmonella* status. However, in the description of the sampled

population of this present study, we noticed that the proportion of the “barn and free-range” category is relatively small (23%). Moreover, the very wide confidence intervals suggest that there might be a problem due to sample size.

Most of the studies have proven vaccination to be an important aid to reduce or possibly eliminate *Salmonella* Enteritidis from laying flocks (Davies and Breslin, 2001b; 2003b; 2004). In the United Kingdom for instance, most of the laying flocks which have been implicated in the recent outbreaks of *Salmonella* Enteritidis in human beings were unvaccinated (Davies and Breslin, 2001). In the present analysis vaccination seemed not to have a significant protective effect. In the cases when *Salmonella* serovars other than *Salmonella enteritidis* are present concurrently in flocks vaccinated for *Salmonella enteritidis*, then considerably more contamination with these other *Salmonella* serovars may occur (Davies and Breslin, 2004). Another explanation why vaccination was less effective than expected, is that hens might have been infected before the vaccination was completed. Therefore it would have been interesting to exploit the period when the flock had been vaccinated as an explanatory variable. Such a variable was indeed available in the initial database but we chose to leave it aside for two main reasons. First, since the variables “vaccination status” and “vaccination period” were related to each other, we used only one of them to avoid multicollinearity problems. Second, from the description of the “vaccination period” variable, we had 88 holdings where vaccination was performed at rearing out of the 90 holdings where hens were vaccinated, leaving us with nothing to properly compare these findings with. Furthermore, effective protection owed to vaccination might occur only when the challenge dose is low. It is crucial to keep in mind that for vaccination to work effectively, an efficient cleaning and disinfection of laying houses between successive flocks is compulsory (Davies and Breslin, 2003b; Van den Bosch 2003). In this study, other factors like hygiene practices or pest control and their potentially

confounding effects on the association between vaccination and the probability of being infected by *Salmonella,* were not taken into account.

The influence of temperature on the growth of *Salmonella* in food has been well documented. It is known that in all countries the incidence of human salmonellosis is highest during the summer (Baird-Parker, 1990; CNRSS, 2004; Kovats *et al*., 2004). Even though a statistically significant effect of the "month" variable is reported from our study, it is difficult to show the direction of the influence as only the month of July had borderline significance. Mollenhorst *et al*, 2005 came to the same conclusion. During the summer season of the year 2003, a large increase of *Salmonella* infections was observed in Belgium and in The Netherlands. This increase could probably be attributed to the extremely hot weather during the summer of 2003. The Dutch study (Van Pelt *et al*., 2004) showed that a concomitant outbreak of *Salmonella* and avian influenza led to a shortage of eggs on the Dutch market, which was to be compensated for with imports, providing a reasonable explanation for this apparent seasonal trend.

This present study showed no evidence of significant differences in the distribution of *Salmonella* among laying flocks according to regional repartition, and the odds ratios were very close to 1 in both faecal and dust samples. Again we should note that the sample repartition is not really equitable, the Walloon holdings representing only 18%. On the other hand, the number of human salmonellosis cases across the country is clearly much higher in Flanders. Although the eggs produced in Belgium do not necessarily tend to be consumed locally, the food practices vary between both regions (CNRSS, 2004; AFSCA, 2006).

The impact of the age factor on the occurrence of *Salmonella* among egg laying flocks cannot really be established here, as the odds ratios and the confidence intervals were all close to 1.

At last, other risk factors which were not considered in the present study are important to mention. For example, it could be useful to build a model taking into account flock characteristics (type of breed, number of flocks on the farm, multi-age farm or not), farm management (control of pest access, visitors allowed or not, feed composition and feeding practices, drinking water), cleaning and disinfecting practices related with the contamination status of the previous flock in the same hen house ((Henzler & Opitz, 1992; Kinde *et al*., 1996; Shirota *et al*., 2000; Garber *et al*., 2003; Liebana *et al*., 2003; Kinde *et al*., 2005). Knowing that non-typhoid *Salmonellae* have very wide host ranges, it is important to take into consideration all various potential vectors surrounding the flock.

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List of Tables

Table 1: Frequency of Salmonella positive/negative flocks (percentage of all 148 flocks) by categorical independent variables and sample type. Association P-values between each categorical variable and the presence/absence of *Salmonella* using Pearson Chi-Square test are shown.

	Dust sample type			Faecel sample type		
Variable	Positive (%)	Negative(%)	Assoc χ^2 P-value	Positive(%)	Negative(%)	Assoc χ^2 P-value
region			0.9698			0.5598
Flanders	38 (25.68)	84 (56.76)		40 (27.03)	82 (55.41)	
Walloon	8 (5.41)	18 (12.16)		7 (4.73)	19 (12.84)	
SamplingTime			0.6570			0.4347
Feb	2 (1.35)	4 (2.70)		3 (2.03)	3 (2.03)	
Mar	4 (2.70)	14 (9.46)		7 (4.73)	11 (7.43)	
Apr	5 (3.38)	16 (10.81)		4 (2.70)	17 (11.49)	
May	7 (4.73)	15 (10.14)		9 (6.08)	13 (8.78)	
Jun	12 (8.11)	16 (10.81)		7 (4.73)	21 (14.19)	
Jul	7 (4.73)	10 (6.76)		8 (5.41)	9 (6.08)	
Aug	4 (2.70)	8 (5.41)		3 (2.03)	9 (6.08)	
Sep	5 (3.38)	19 (12.84)		6 (4.05)	18 (12.16)	
Production Type			<0.0001			0.0002
Cage	45 (30.41)	69 (46.62)		45 (30.41)	69 (46.62)	
barn & free range	1 (0.67)	33 (22.30)		2 (1.35)	32 (21.62)	
Vaccination Status			0.0260			0.0573
Yes	22 (14.86)	68 (45.95)		22 (14.86)	68 (45.95)	
No	22 (14.86)	26 (17.57)		21 (14.19)	27 (18.24)	
Unknown	2 (1.35)	8 (5.41)		4 (2.70)	6 (4.05)	

Table 2: Estimated *Salmonella* infection odds ratios (95% confidence interval limits) and p-values from univariate simple logistic models (USLM) and univariate multiple logistic models (UMLM) under Independency and the bivariate multiple logistic model (BMLM) using GEE approach assuming an exchangeable working correlation between the outcomes. Significant risk factors ($p < 0.05$) are denoted by (*) while the borderline ($0.05 \leq p < 0.1$) risk factors with (+).

1	2		3		4	
	USLM	USLM	UMLM	UMLM	BMLM using GEE	
COVARIATE	DUST	FAECES	DUST	FAECES	DUST	FAECES
Sampling time						
February vs september	1.90	3.00	0.95	1.60	0.61	1.11
	(0.27-13.52)	(0.47-19.04)	(0.11-8.39)	(0.20-12.78)	(0.03-10.89)	(0.13-9.22)
	0.522	0.244	0.965	0.660	0.738	0.925
March vs september	1.09	1.91	0.70	1.24	0.70	1.28
	(0.25-4.79)	(0.51-7.17)	(0.14-3.61)	(0.27-5.63)	(0.12-4.14)	(0.27-6.13)
	0.914	0.338	0.669	0.780	0.697	0.758
April vs september	1.19	0.71	0.71	0.35	0.73	0.35
	(0.29-4.85)	(0.17-2.94)	(0.15-3.35)	(0.07-1.76)	(0.15-3.70)	(0.06-1.97)
	0.811	0.633	0.662	0.203	0.709	0.232
May vs september	1.77	2.08	1.41	2.22	1.65	2.56
	(0.47-6.72)	(0.59-7.29)	(0.28-7.20)	(0.45-10.92)	(0.36-7.60)	(0.44-15.03)
	0.399	0.254	0.677	0.326	0.520	0.298
June vs September	2.85	1.00	2.83	0.74	3.04	0.70
	(0.83-9.82)	(0.28-3.52)	(0.67-12.01)	(0.17-3.21)	(0.69-13.35)	(0.14-3.47)
	0.097	1.00	0.157	0.691	0.142	0.664
July vs september	2.66	2.67	3.53	3.32	4.89+	3.68
	(0.67-10.57)	(0.71-10.05)	(0.66-18.75)	(0.65-17.05)	(0.83-28.96)	(0.58-23.45)
	0.165	0.147	0.139	0.150	0.080	0.169
August vs September	1.90	1.00	1.92	0.64	1.97	0.62
	(0.40-8.98)	(0.20-4.95)	(0.32-11.39)	(0.10-4.00)	(0.27-14.21)	(0.09-4.28)
	0.418	1.000	0.475	0.634	0.503	0.627
Production type						
Cage vs barn and free-range	21.52*	10.43*	16.38*	7.88*	20.11*	10.27*
	(2.84-162.98)	(2.38-45.70)	(1.92-139.99)	(1.47-42.12)	(2.52-160.49)	(2.13-49.57)
	0.003	0.002	0.011	0.016	0.005	0.004
Vaccination status						
Vaccination vs no vaccination	0.38*	0.42*	0.50	0.70	0.49	0.69
	(0.18-0.80)	(0.20-0.88)	(0.20-1.28)	(0.27-1.83)	(0.18-1.31)	(0.23-2.02)
	0.011	0.021	0.148	0.463	0.154	0.494
Vaccination unknown vs no vaccination	0.30	0.86	0.35	1.81	0.23	1.70
	(0.06-1.54)	(0.21-3.43)	(0.05-2.23)	(0.34-9.65)	(0.02-2.06)	(0.37-7.89)
	0.148	0.828	0.266	0.486	0.188	0.498
Age						
Age	1.03+	1.04*	1.02	1.04*	1.02	1.03+
	(1.00-1.06)	(1.01-1.08)	(0.98-1.05)	(1.00-1.08)	(0.98-1.06)	(1.00-1.07)
	0.067	0.008	0.291	0.027	0.439	0.050
Flocksize						
Flocksize	1.00*	1.00*	1.00+	1.00*	1.00+	1.00+
	(1.00-1.00)	(1.00-1.00)	(1.00-1.00)	(1.00-1.00)	(1.00-1.00)	(1.00-1.00)
	0.002	0.001	0.081	0.030	0.049	0.071

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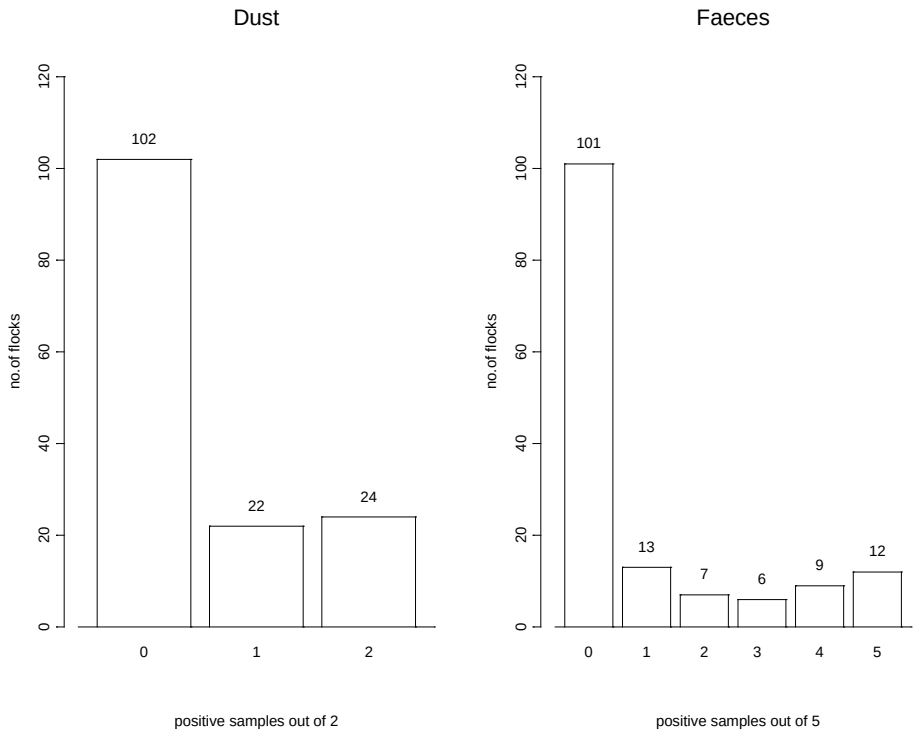

Figure 1: number of flocks and the frequency of samples, out of the two for dust and out of five for faeces, which tested positive for *Salmonella*.

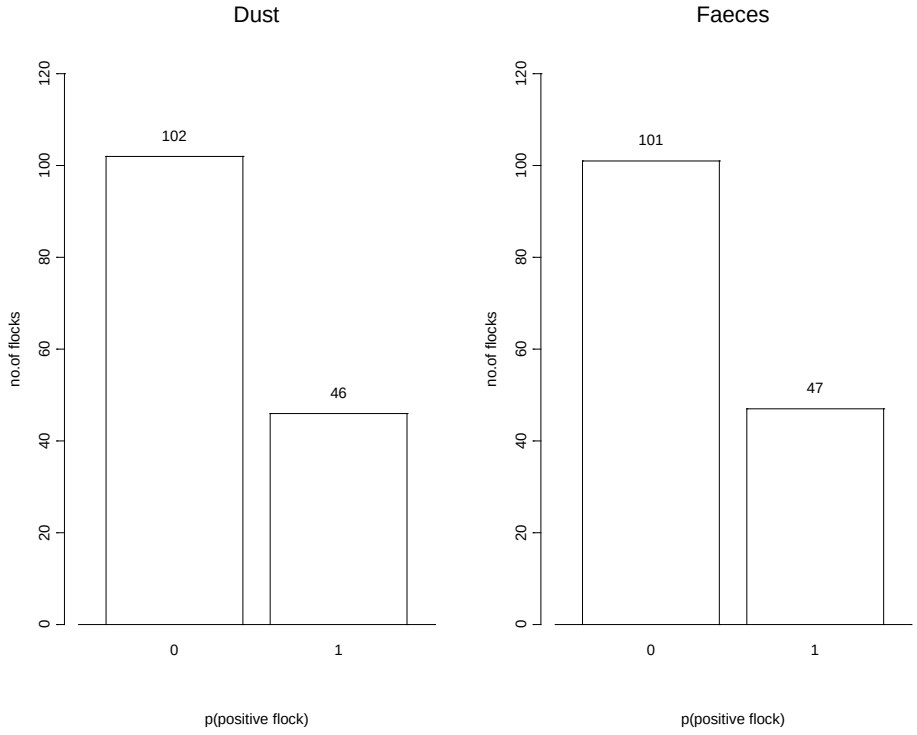

Figure 2: number of flocks and their state of salmonella, 0 for absence and 1 for presence of *Salmonella* after collapse of figure 1.