Royal Veterinary College
University of London

FSA strategic priority: Epidemiological analysis of *Campylobacter* data generated in an industry biosecurity study

Milen Georgiev
Wendy Beauvais
Jane Downes
Javier Guitian

22 November 2014

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EXECUTIVE SUMMARY

The aim of this study was to analyse information collected by the industry on *Campylobacter* colonisation of poultry batches originating from farms with enhanced biosecurity and from control farms with standard biosecurity. The study supports the activities of the FSA and the Joint Working Group on *Campylobacter* (JWG) aimed at reducing levels of *Campylobacter* spp. colonisation in poultry at farm level in the UK.

The hypothesis that enhanced farm biosecurity contributes to a decrease of the risk of *Campylobacter* colonization at high levels (≥1,000 colony forming units per gram [cfu/g] in caeca) was tested. Furthermore, the contribution of partial depopulation, empty days between flocks in the sheds, type of hybrid and season to the probability of batch colonisation with *Campylobacter* at high levels was quantified. Finally, the data were used to compare the effect on our results of assessing batch status by means of pooled caecal samples vs. pooled neck skin samples. The analysis includes 1686 batches originating from 16 'model' farms with elevated biosecurity and 429 batches grown in farms with standard biosecurity between September 2011 and August 2013. For each batch, data were collected on selected factors / characteristics and the levels of *Campylobacter* spp. were measured in pooled caecal and pooled neck skin samples. All samples were tested according to ISO10272-2 2006. The results of microbial testing of caecal samples were used to classify batches according to the same threshold targets agreed by the JWG and applied by FSA, for monitoring at neck skin samples, in three bands: Low (<100cfu/g), Medium (100 to <1,000cfu/g) and High (≥1,000cfu/g).

Following the identification of two suitable control groups of farms with standard biosecurity from the datasets provided by the industry, statistical analyses were carried out for one factor at a time (univariate); and adjusting for confounding factors (multivariate), to assess the relationship between selected 'on farm' factors and the probability that the batch was highly colonized. The findings support the following conclusions:

- A substantial risk of *Campylobacter* spp. infection is present early in the food chain as a large proportion of all the poultry batches included in this study (>60%) were colonized in caeca at high levels.
- The risk of batch colonisation exhibits seasonality, with a peak in summer when almost 100% of the studied batches were colonized.
- Enhancement of biosecurity in commercial poultry farms can contribute to the reduction of batch colonisation at thinning to 64% of the risk in control farms. Following thinning the potential effect of increased biosecurity becomes much less apparent.

- In addition to the season, husbandry factors such as the practice of partial depopulation (thinning), hybrid type and empty period between flocks in a shed were also associated with the probability of batch colonisation at high levels ≥ 1,000cfu/g:
    - In farms with enhanced biosecurity, batches in which thinning had been previously carried out were significantly more likely to be heavily colonized than batches in which thinning had not been practised (77.9% vs. 58.9%).
    - Hybrid types were associated with the risk of colonisation; batches of the Ross 308 hybrid experienced the lowest risk of colonisation (58.3%). Two other hybrids were also less likely to be colonized (69.9% for Ross 708 and 68.8% for Cobb) when compared with more than 77.0% for the rest of the hybrids R, C&R and JA.
    - An empty period of more than one week between the flocks increased the risk of colonisation by 16% compared with the risk if empty period was up to 1 week.
- Under the assumption that the results obtained in the study farms can be extrapolated to the general population of batches sent to slaughterhouses and that no major confounders have been omitted, estimates of the potential impact of specific interventions targeting the risk factors under investigation have been obtained:
    - The introduction of enhanced biosecurity in all farms could result in a 26% reduction in the proportion of highly colonized batches sent to slaughterhouses.
    - If none of the batches were subject to thinning, 24% of highly colonized batches would be prevented.
    - If all batches were of the hybrid types of lower risk, between 17% and 33% of batch colonisation would be prevented

BACKGROUND

Campylobacter spp. are spiral-shaped thermo- and microaerophilic bacteria that are recognized as a cause of food-borne illness affecting around 280,000 people with approximately 1,000 hospitalizations and 100 deaths in the UK per year. The Food Standards Agency (FSA) considers the situation as unacceptable (FSA, 2010) given the high impact on public health, with an estimated cost to the UK economy of approximately £900 million per year.

Campylobacter spp. have been isolated in poultry (Allen et al., 2008), other birds (Yogasundram et al., 1989), livestock (Keller et al., 2007; Ellis-Iversen et al., 2009) and humans (Tam et al., 2003). *Campylobacter jejuni* is the species most frequently identified in human cases, although other species such as *Campylobacter coli* and *Campylobacter lari,* are also isolated. Many infections in people are asymptomatic but the ingestion of as few as 500 *Campylobacter jejuni* has been shown to be sufficient to cause disease in humans (Robinson, 1981; Black et al., 1988). The course of the disease varies in severity from three to six days of diarrhoea to development of complications, including pancreatitis, arthritis and neurological disorders (Heymann, 2008). Poultry meat has been identified as a key risk factor for human campylobacteriosis (Harris et al., 1986), and the intestines of commercial broilers (*Gallus gallus*) are known to be often colonized (Bull et al., 2008; McDowell et al., 2008). The utilization of microbial genetic data has provided further evidence of linkages between *Campylobacter* strains in poultry and humans (Sheppard et al., 2009; Muellner et al., 2013).

The pathogen may be introduced from the environment (Bull et al., 2006; Ellis-Iversen et al., 2012) to a poultry house via different routes including houseflies (Shane et al., 1985), farmers' boots during daily operations or staff during partial depopulation (Allen et al., 2008). Once *Campylobacter* infection is introduced to a few individuals in a broiler flock, further horizontal transmission occurs from infected individuals to the surrounding environment and to other susceptible birds (Herrocks et al., 2009; Newell et al., 2011), with potential for increase in pathogen virulence after several passages (Sang et al., 1989) and colonisation (presence of *Campylobacter* spp. in birds' intestine) of the entire flock in a matter of a few days (Shreeve et al., 2002). Although young chickens are susceptible to *Campylobacter*, colonisation of commercial broilers usually occurs after day 14 of their production cycle (Bull et al., 2006).

The European Food Safety Authority (EFSA) has estimated that 20% to 30% of campylobacteriosis in humans may be attributed to the consumption of broiler meat, and 50% to 80% to the chicken reservoir as a whole (EFSA, 2010b). A baseline survey carried out by EFSA across 26 EU countries and two other countries in Europe in 2008 (EFSA, 2010a) showed a high prevalence of poultry batches testing positive at slaughter: 71.2 % on average (95% C.I. 68.5% - 73.7%). The prevalence in the UK was estimated as 75.3 % (95%C.I. 69.9% - 80.1%) based on 401 sampled batches after accounting for clustering of batches within farms (EFSA, 2010a; Powell et al., 2012).

The EFSA experts have concluded that *Campylobacter* spp. has the highest public health relevance of all the diseases to be considered in meat inspection of poultry (Ricci Antonia, 2014). In the UK, a step approach of reducing *Campylobacter* to target thresholds was agreed among members of the JWG, including poultry growers and processors, FSA, Defra, the British Poultry Council, the British Retail Consortium and the National Farmers Union (FSA, 2010). For simplicity in the monitoring process, *Campylobacter* counts were grouped into three bands: <100 colony forming units per gram (cfu/g), 100 to <1,000 cfu/g, and ≥1,000 cfu/g in neck skin samples. Routine monitoring on those samples commissioned by FSA indicates that 30.0% of poultry in 2012 and 31.0% a year later were still in the highest band compared with 27.0% in 2008 (FSA, 2013).

Good hygiene practices including additional decontamination treatments could support rapid reduction in the number of *Campylobacter* on poultry carcasses (Shane, 2000). However, a lack of public confidence in such interventions was identified in a survey of consumers' attitudes (GfK, 2013).

Several 'on farm' options have been investigated to reduce potential transmission including the use of chlorinated drinking water (Ellis-Iversen et al., 2009), bacteriophage therapy (Wagenaar et al., 2005) and bacteriocins (Stern et al., 2008) or the use of probiotics (Willis and Reid, 2008) and vaccination (Buckley et al., 2010) to increase poultry resistance to infection. However, many of those are still in development or considered as not feasible at the moment.

The identification of farm-level factors associated with the risk of colonisation can support the implementation of changes to existing husbandry practices in order to reduce consumer exposure. A protective effect of farm hygiene measures on batch colonisation was suggested in studies in the Netherlands (Van de Giessen et al., 1996), the UK (Gibbens et al., 2001) and Denmark (Rosenquist et al., 2013). Other authors challenge the effectiveness of these options at primary production, as having limited and unpredictable effect (Wagenaar et al., 2006). A comprehensive literature review on 'on farm' measures with relevance for UK (Newell et al., 2011) indicated that, theoretically, enhanced biosecurity in commercial farms may contribute to a reduction in batch colonisation.

Studies of biosecurity at farm level are part of the JWG Action plan to investigate the potential to reduce prevalence at flock level and *Campylobacter* batch colonisation at primary production level. Consequently, the industry has tested the effectiveness of enhanced biosecurity (i.e. operating in each poultry house (shed) as a bio-secure unit, using protective clothes and shed- specific equipment in addition to standard procedures) on a number of 'model farms' between September 2011 and August 2013.

The work presented in this report is an epidemiological analysis of the data generated by this intervention, including the formal comparison of the levels of *Campylobacter* caecal colonisation in batches raised in 'model farms' under enhanced biosecurity with

two groups of control batches from farms with ‘standard biosecurity’. The report compiles information and results of the data analyses in line with JWG activities as a part of the industry commitment to reduce the proportion of highly colonized batches.

1. AIMS AND OBJECTIVES

The aim of this study was to analyse the data generated by the industry on *Campylobacter* colonisation of poultry batches originating from farms with enhanced biosecurity and from control farms with standard biosecurity. The data generated by the project has been analysed in order to achieve the following objectives:

- ✓ To test the hypothesis that biosecurity 'on-farm' contributes to a decrease in *Campylobacter* colonisation at high level (≥1,000cfu/g in caeca), comparing model farms in which biosecurity was enhanced with control farms with 'standard' biosecurity.
- ✓ To assess the relationship between selected husbandry factors and the likelihood of *Campylobacter* colonisation at high level (≥1,000cfu/g in caeca).
- ✓ To compare the results of assessing the status of batches by means of testing pooled caecal samples vs. pooled neck skin samples.

The analysis was carried out using data from batches originating from 16 'model farms' with elevated biosecurity belonging to 5 companies during the period September 2011 - August 2013. These data were compared with: i) batches grown in farms with standard biosecurity and slaughtered by four of the companies between March 2012 and October 2013 and ii) batches in five other farms selected for having 'similar conditions' to model farms for the period January 2013 – August 2013. Data were recorded at slaughterhouse level and included information on husbandry factors attributed to the batch. For all the studied batches, the level of *Campylobacter* spp. was measured in pooled caecal samples and pooled neck skin samples.

2. MATERIALS AND METHODS

2.1. Data sources and study population

Data were generated by the industry as one set of data for farms with enhanced biosecurity ('model' farms) and two sets of data for farms with standard biosecurity ('control farms'). The potential use of FSA data from national monitoring was considered, but it was decided to omit these data for purpose of this study as the nature of these data made it not directly comparable with the available industry data in terms of time period, grouping of batches and time of slaughter.

2.1.1. Model farms

Sixteen farms were selected by the industry represented in JWG to be 'model' examples, where a new protocol for enhanced biosecurity was implemented since August 2011. Although no formal probabilistic selection of candidate farms for enhancement of biosecurity was conducted, the 16 farms were considered to be representative of current production practices in the UK, were geographically dispersed and belonged to five different companies. Farm staff was trained and operated each poultry house (shed) as a bio-secure unit using protective clothes and shed-specific equipment in addition to standard procedures. Full details on applied biosecurity measures in model farms are available in ANNEX A (Table 8, Table 9). Model farms were located in England, Wales, Scotland and Northern Ireland and linked to different retailers. The volume of poultry production varied among the farms as the number of sheds ranged from 1 to 12 per farm.

2.1.2. Control farms

Two groups of control farms were investigated:

In group one (control farms 1), the batches were selected by four poultry companies (abbreviated as Q, R, S, T) when present at the slaughterhouses they manage. Broilers originated from different farms where standard biosecurity was applied (assured chicken production ACP standards and compliance with the Red Tractor assurance scheme). Information on the location, volume of the production or size of the flocks was not available. Group two (control farms 2) consists of batches originating from five farms with similar conditions to five of the model farms, purposively selected by the industry.

2.2. Study design

The study was conducted in the UK, between 1 September 2011 and 31 August 2013, as a batch-level investigation of risk factors for caecal colonisation at high level (≥1000cfu/g), including a comparison of the risk of high level colonisation at caeca between model and control farms. The results in caeca were compared with the results in pooled neck skin samples to assess the correlation between the two measurements. Batches of chickens (birds which had been grown in the same shed and

delivered to a slaughterhouse on one single day) were selected as the study unit. The measured outcome was level of batch colonisation.

Data were collected for 1,686 batches from model farms. Batches were selected from one shed if the farm consisted of fewer than 5 sheds or from two sheds otherwise. The 2-year study period was divided in 16 intervals of 45 days (a month and a half) each and each batch allocated to one of the 16 intervals. For batches grown in model farms, information was also obtained on other husbandry factors which could potentially have an influence upon colonisation of broilers, namely:

- Welfare status, defined as 'enhanced' when broilers can be reared in the flock up to 39kg live weight on one squared meter of useable area or ''standard' when maximum stocking density is 33kg/m^2) (available for 1,620 batches, 96.0% of the total).
- Number of empty days between flocks (available for 1,426 batches, 84.6%).
- Number of days from partial depopulation (thinning) to the end of the production cycle (available for 1,452 batches, 86.1%).
- Type of broiler hybrid (available for 1,154 batches, 68.4%).

The batches from the first group of control farms (control farms 1) were produced and processed by four companies but the exact farm of origin of each batch was not recorded. Consequently, an assumption was made that records for each of the four companies represent the situation in four large farms without identification of the individual poultry sheds. Data were collected for the period 16 April 2012 to 15 October 2013 and 366 batches were considered as eligible for comparison with those from model farms.

The five farms in the second control group (control farms 2) were recorded to match five model farms for all factors except biosecurity. Thirty batches originating from one shed per CF2 farm were investigated between 16 January 2013 and 31 August 2013.

The characteristics of each dataset and the number of tested batches are summarized in Table 1.

2.3. Samples and laboratory testing

For each of the study batches, samples were taken from the caeca of five birds in the batch and pooled as a single sample. The five birds were selected as non-consecutive carcasses on the slaughter line. These samples are considered to represent the situation in the whole batch. All samples were tested to enumerate *Campylobacter* according to agreed standards of International Organization for Standardization (ISO) ISO10272-2 2006. The results were recorded at batch level and in 16 sampling periods, each one covering a month and a half.

2.4. Data analysis

2.4.1. Data management and descriptive statistics

The results of laboratory testing of caecal samples were classified according to the same threshold targets agreed by the JWG and applied by FSA for monitoring of results of neck skin samples in three bands Low (<100cfu/g) Medium (100 to <1,000cfu/g) and High (≥1,000cfu/g) for all tested batches. Batch colonisation across the study period was described and graphically presented. Descriptive statistics showed that the vast majority of batches belonged to the low and high categories and it was decided to conduct all statistical analyses for high (≥1,000cfu/g in caeca) vs. low or medium (<1,000cfu/g in caeca) batches (i.e. low and medium categories were collapsed).

2.4.2. Statistical analysis

The risk of being a highly colonized batch was estimated for:

- Batches raised under enhanced biosecurity vs. control batches raised under standard biosecurity.
- Batches harvested at thinning (partial depopulation) vs. at the end of the cycle (depopulation).
- Batches composed of different hybrids: (Cobb, C&R, R, Ross 308, Ross 708 and JA).
- Batches with different empty days before the start of the cycle: (1-7, 8-14, 15-21 and 21-47),
- Batches with different number of days between thinning and depopulation: (0-2, 3-4, 5-6, 7-11, 12-14 and 15-21).
- Batches for which welfare was 'standard' or 'enhanced'.
- Batches which were slaughtered at each of the 16 45-day intervals between 1st September 2011 and 31st August 2012.

Comparisons were first carried out for each factor independently (univariate). This was followed by multivariate analysis to explore the combined effect of multiple factors on colonisation. Three multivariate models were built:

- Model 1: a random effects logistic regression model (Regression Model 1) for batches originating from model farms vs. those from control farms. This model was used to compare the odds of colonisation (ratio of the number of highly colonized batches vs. number of batches that are less colonised <1000cfu/g) between farms with enhanced biosecurity (model farms) and farms with standard biosecurity (control farms 1). The model allowed simultaneous consideration of the potential effect of other variables (harvest occasion and season) and accounted for the fact that batches from the same farm may be more "similar" than batches from different farms (i.e. within-farm clustering).

- Model 2: a random effects logistic regression model (Regression Model 2) for batches originating from model farms but raised under different husbandry practices. This model was limited to batches from model farms because data on husbandry factors are available in model farms only. The model compares the odds of colonisation (ratio of the number of highly colonized batches vs. number of batches that are less colonised <1000cfu/g) between batches at different harvest occasion (thinning or depopulation) while simultaneously considering the potential effect of other variables (type of hybrid, empty days between flocks and season). As for model 1, model 2 also accounted for the fact that batches from the same farm may be more "similar" than batches from different farms (within-farm clustering).
- Model 3: a random effects logistic regression model (Regression Model 3) for batches originating from model farms and comparing the odds of colonisation (ratio of the number of highly colonized batches vs. number of batches that are less colonised <1000cfu/g) at depopulation between batches where partial depopulation was conducted and batches without partial depopulation. This model considered the potential effect of season and, as the previous 2 models, accounted for the fact that batches from the same farm may be more "similar" than batches from different farms (within-farm clustering).

The relationship between the results obtained from the testing of pooled caecal samples and pooled neck skin samples was assessed by cross-tabulation, estimating the proportions of batches positive or negative by caecal sampling that would have been classified as positive or negative by pooled neck sampling.

These univariate and multivariate statistical methods are described in detail in Annex B.

2.4.3. Estimation of Population Attributable Fraction (PAF)

Estimates of the strength of the association between i) enhanced biosecurity, ii) partial depopulation and iii) hybrid type with odds of colonisation at high levels (obtained from the models mentioned above), were used to estimate the proportion of heavily colonized batches that could be attributed to each of these factors; the Population Attributable Fractions (PAFs). To provide an indication of the potential effect of reducing the exposure of the UK broiler population to those factors, the proportion of heavily colonized batches that would be prevented was estimated under the following different scenarios: i) enhancement of biosecurity ii) elimination of the practice of thinning and iii) use of low-risk hybrid types. For this calculation assumptions were made as to the total population of the UK broiler population currently "exposed" to each of the 3 individual factors.

3. RESULTS

3.1. *Campylobacter* colonisation of the study batches and seasonality

The status of all studied batches with respect to *Campylobacter* based on the testing of pooled caecal samples are presented in Table 1 and Table 2. Overall, 70.3% of all the studied batches were heavily colonized (≥1,000 cfu/g in pooled caecal samples). The proportion of infected batches exhibited a seasonal pattern, with peaks during the summer period (Figure 1).

Table 1 Structure and contents of the combined data sets of farms with enhanced biosecurity (Model Farms) and standard biosecurity (Control Farms) used in the epidemiological analysis of *Campylobacter* data generated in the industry biosecurity project (UK, 2011-2013)

Farms	Biosecurity	Period during which sampling was undertaken (1.5 month periods, from August 2011 (period 1) to September 2013 (period 16)	Number of farms	Sheds per farm (total)	Batch harvest type (thinning or depopulation)	Total number of batches where caecal samples were obtained at thinning or depopulation	Number of batches sampled at both thinning and depopulation and (number sampled only once)	Other factors for which information was available
Model farms	Enhanced	1-16	16	1-12 (98)	Thinning Depopulation	803 883	720 (83) 720 (163)	▪ Welfare level (Enhanced/Standard) ▪ Empty days (1-47) ▪ Days to depopulation (1-21) ▪ Hybrid type (Cobb, C&R, R, Ross308, Ross708, JA)
Control farms 1	Standard	6-17	na[i]	Na	Thinning Depopulation	199 200	118 (81) 118 (82)	Companies (4)
Control farms 2	Standard	12-16	5	1 (5)	Thinning Depopulation	16 14	12 (4) 12 (2)	Declared similarity to 5 model farms

[i] na: information is not available

Table 2 Results of testing pooled caecal samples from batches included in the epidemiological analysis of *Campylobacter* data generated in the industry biosecurity project (UK, 2011-2013)

Bands of the results	at thinning			at depopulation			
	Control farms 1	Control farms 2	Model Farms	Control farms 1	Control farms 2	Model Farms	TOTAL
1 to<100cfu/g	21 (10.6%)	4 (25.0%)	339 (42.2%)	23 (11.5%)	0	176 (19.9%)	**563 (26.6%)**
100 to <1,000 cfu/g	1 (0.5%)	0	26 (3.2%)	0	1 (7.2%)	37 (4.2%)	**65 (3.1%)**
≥1,000 cfu/g	177 (88.9%)	12 (75.0%)	438 (54.5%)	177 (88.5%)	13 (92.8%)	670 (75.9%)	**1,487 (70.3%)**
TOTAL	**199**	**16**	**803**	**200**	**14**	**883**	**2,115**

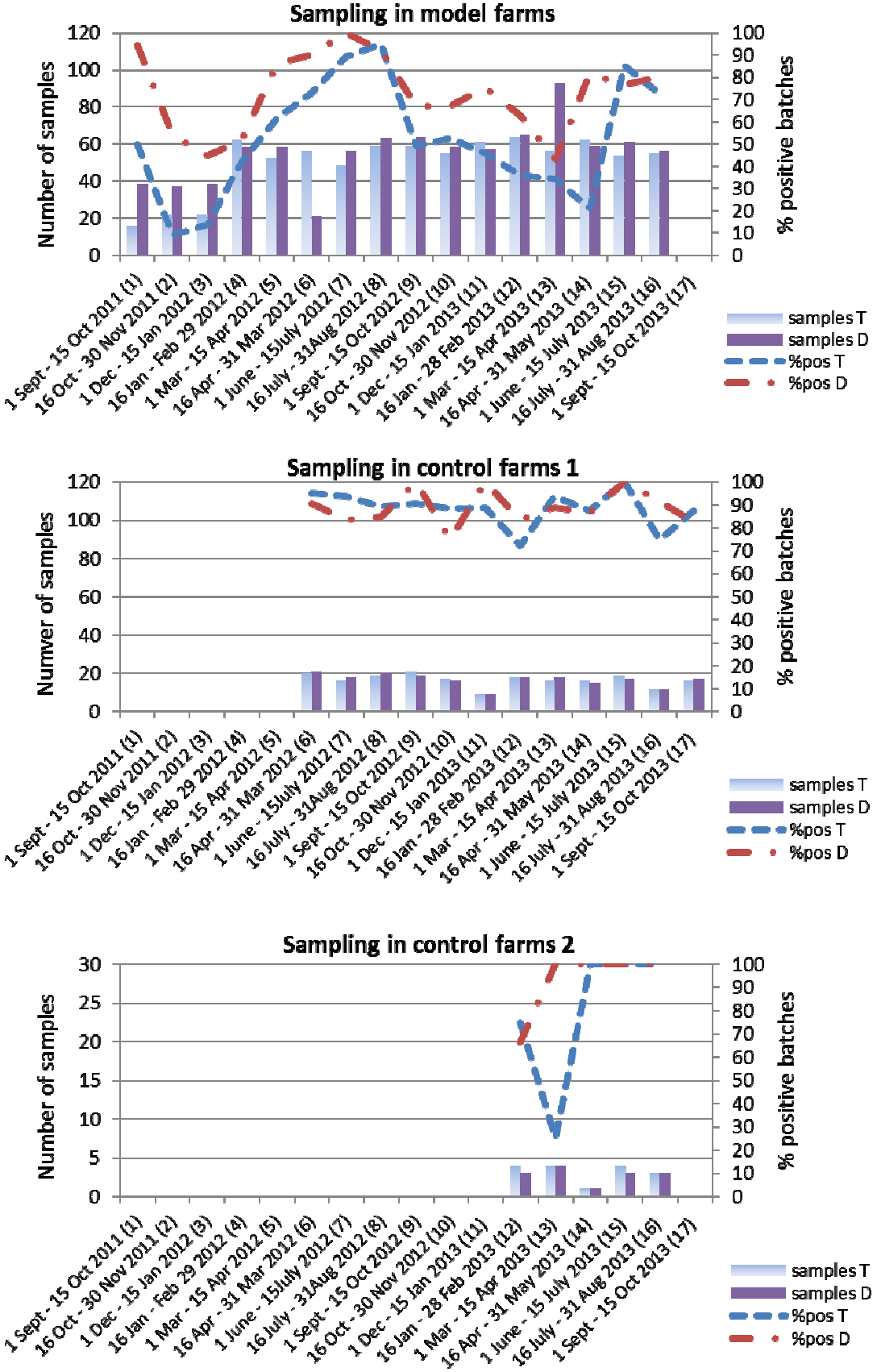

Figure 1 Seasonal variation in *Campylobacter* colonisation of batches in model farms and control farms. Colonized batches are those with ≥1,000 cfu/g in pooled caecal samples obtained either at thinning (T) or at depopulation (D).

The relationship between the results obtained from the testing of pooled caecal samples and pooled neck skin samples is presented in Table 3. Data show that 91 % of negative batches in caeca are also identified as negative at neck skin samples, 40% of positive batches in caeca are positive in neck skin samples and 11 % of positive batches in neck skin samples are negative in caeca. Considering samples from caeca as the 'gold standard', the use of neck skin samples will have a sensitivity of 40% and a specificity of 91% to correctly identify batch colonisation at primary production.

Table 3 Association between results in caecal and neck skin samples of batches. Results from a total of 1378 batches sampled between 1 September 2011 and 31st August 2013

		Caeca		
		Positive ≥ 1000 cfu/g	**Negative ≤ 1000 cfu/g**	TOTAL
Neck skin	**Positive ≥ 1000 cfu/g**	359	43	402
	Negative ≤ 1000 cfu/g	548	428	976
	TOTAL	907	471	**1,378**

3.2. Relationship between individual risk factors and batch colonisation (univariate analysis)

When analysed individually (i.e. analysis not adjusted for confounding), all the factors under study, except the poultry company of origin, were significantly associated with the risk of a batch being colonized at high levels. The results of this initial analysis are presented in

Table 4.

- Batches harvested at thinning are significantly less likely to be heavily colonized than those harvested at the end of the cycle: Approximately 3 out of 5 batches harvested at thinning were colonized at high level while at the end of the cycle 4 out of 5 batches were colonized.
- Batches grown under enhanced biosecurity were significantly less likely to be colonized than batches raised under standard biosecurity (65.7% in model farms, 88.4% control group 1 and 88.3% in control group 2).
- Batches from model farms which are reared under enhanced welfare, allowing for increase in stocking density to 39 kg/m^2, were significantly more likely to be colonized at a high level than those raised under standard welfare and lower density (colonisation of 70.6% under enhanced welfare vs. 60.7% in standard welfare).
- Batches of different hybrids grown in model farms had significantly different levels of batch colonisation (≥1000 cfu/g). Batches of the Ross 308 hybrid experienced the lowest risk of colonisation (58.3%). Two other hybrids also showed lower risk of colonization (69.9% for Ross 708 and 68.8% for Cobb) when compared with more than 77.0% for the rest of the hybrids.
- Batches from model farms had significantly different levels of batch colonisation (≥1000 cfu/g) depending on the duration of empty periods between flocks. Batches grown after an empty period of 2-3 weeks had the lowest risk of colonisation (60.0 %).
- The number of days between thinning and depopulation in model farms significantly affected the risk of batch colonization (≥1000 cfu/g). Batches grown in farms with 3-4 days between partial and final depopulation experienced the lowest risk of colonisation (57.5%) and those in which thinning took place 12 to 14 days before depopulation the highest (85.8%).
- Batches produced in 'control farms 1' had similar levels of colonisation amongst the four different companies.
- At depopulation, batches from model farms were significantly less likely to be colonised (≥1000 cfu/g) if thinning had not been applied (58.9 %) than when thinning had been practised (77.9%).

Table 4 Univariate associations between potential risk factors and *Campylobacter* colonisation at high level (≥1,000 cfu/g in pooled caecal samples). Results from a total of 1,378 batches sampled between 1 September 2011 and 31st August 2013.

Variable	Categories	Number (%) of Batches ≥1,000cfu/g	Number of Batches <1,000cfu/g	p-value (chi^2)[i]
Harvest occasion	Thinning	627 (62.2)	381	<0.001
	Depopulation	860 (78.4)	237	
Biosecurity	Model Farms	1,108 (65.7)	578	<0.001
	Control farms 1	344 (88.4)	45	
	Control farms 2	25 (88.3)	5	
Welfare in model farms	Enhanced	560(70.6)	233	<0.001
	Standard	502 (60.7)	325	
Hybrid in model farms	Cobb	159 (68.8)	72	<0.001
	R	436 (77.3)	128	
	C & R	16 (94.1)	1	
	Ross 308	126 (58.3)	90	
	Ross 708	58 (69.9)	25	
	JA	37 (90.2)	4	
Empty days in model farms	1-7 days	230 (68.0)	108	0.015
	8-14 days	620 (66.5)	313	
	15-21 days	66(60.0)	44	
	22-47 days	37 (82.2)	8	
	na[ii]	155(59.6)	105	
Days from thinning to depopulation in model farms	0-2 days	64 (71.9)	25	0.001
	3-4 days	191 (57.5)	141	
	5-6 days	321 (65.5)	169	
	7-11 days	275 (67.6)	132	
	12-14 days	91 (85.8)	15	
	15-21 days	22 (78.6)	6	
Companies dealing with batches of	Q	61 (87.1)	9	0.886
	R	66 (90.4)	7	
	S	106 (87.6)	15	

control farms 1	T	121 (89.6)	14	
Practice of partial depopulation in model farms	Thinning had been practised	614 (77.9)	174	<0.001
	Thinning had not been practised	56 (58.9)	39	

[i] chi2 test on (r x c) tables

[ii] information is not available

Due to the low number of batches included the second control group, it was decided to omit this group (control farms 2) from subsequent analysis.

3.3. Multivariate analysis

Table 5 presents the results of a random effects logistic regression (Regression Model 1) comparing the odds of colonisation between farms with enhanced biosecurity (model farms) and farms with standard biosecurity (control farms 1) while considering the potential effect of other variables. Therefore the model provides estimates of the association between biosecurity and colonisation adjusted for potential confounding by harvest occasion and season. The results of the model show that:

- The enhancement of biosecurity and the harvest occasion are significantly associated with the risk of a batch being contaminated and the effects of these two factors are related (i.e. enhanced biosecurity does not have the same effect at thinning than at depopulation and harvesting at thinning vs. at depopulation does not have the same effect on batches with enhanced biosecurity than on batches with standard biosecurity). Enhancement of biosecurity reduces significantly the odds of colonisation when harvesting takes place at thinning (16% of the odds of infection of a standard biosecurity batch harvested at thinning) but the effect is markedly reduced (and statistically not significant) when harvesting takes place at the end of the cycle (57% of the odds of a standard biosecurity batch harvested at depopulation). Harvest occasion is much less important for batches raised under standard biosecurity than batches raised under enhanced biosecurity. A very high proportion (89.1%) of batches raised under standard biosecurity was already colonized at the time of thinning. On the other hand, only 58.3% of batches raised under enhanced biosecurity were colonized at thinning and this proportion increases significantly to 79.1% when harvesting takes place at depopulation.
- The model results confirm also the role of season. The likelihood of batch colonisation is higher in the summer, particularly after 1st June. Using winter 2013 as a reference, the odds of colonisation was 9.76 times higher in the summer of 2012 and 3.38 times higher in the summer of 2013.

Table 5 Results of a random effects logistic regression (Regression Model 1) of enhanced biosecurity and other factors on batch colonisation (defined as ≥1,000 cfu/g in pooled caecal samples). Results from a total of 1,649 batches sampled between 16th April 2012 and 31st August 2013[1].

Factors	OR[i]	P-value [ii]	95% C.I. [iii]	
Biosecurity				
Standard (control farms 1)	1.00	<0.001		
Enhanced (model farms)	0.16		0.07	0.36
Harvest occasion				
Thinning (T)	1.00	0.781		
Depopulation (D)	0.90		0.42	1.93
Interaction between biosecurity & harvest occasion				
Model farm & Depopulation	3.49	0.003	1.55	7.88
Effect of Depopulation:				
- in model farm	3.13		2.39	4.11
- in control farms1	0.90		0.42	1.93
Effect of enhanced biosecurity				
- at thinning	0.16		0.07	0.36
- at depopulation	0.57		0.25	1.27
Sampling period				
16 Apr – 31 May 2012	4.11	<0.001	2.41	7.43
1 June – 31 Aug 2012	9.76	<0.001	5.79	16.46
1 Sept - 30 Nov 2012	1.28	0.180	0.89	1.85
1 Dec - 28 Feb 2013	1.00			
1 Mar - 31 May 2013	0.97	0.876	0.68	1.39
1 June - 31 Aug 2013	3.38	<0.001	2.24	5.08
Constant	4.94	<0.001	2.30	10.59
standard deviation of random effects	0.52		0.33	0.81
Interclass correlation coefficient (rho)	0.08		0.03	0.17

[i] Odds ratio (OR) is a ratio of odds (a ratio of highly colonized batches vs. those that are less colonised <1000cfu/g) in a category of interest vs. odds in baseline category (i.e. OR is a ratio of other two ratios). The OR quantifies how strongly the presence of particular factor is associated with high colonisation in studied population.

[ii] The p-value is an indication of probability, with a value ranging from zero to one, of obtaining a test statistic result at least as large as the one that was actually observed, if there is no differences between compared groups.

[iii] The 95% confidence interval (95% CI) is an interval estimate of a parameter with 95% confidence. We are 95% confident that the true value of the parameter is in our confidence interval.

Likelihood-ratio statistic for comparison with a logistic model not adjusting for clustering (i.e. if rho=0): chibar2(01) = 48.64 Prob > chi2 <0.001

[1] Only batches sampled during this period included as it was the period of overlap in recruitment of batches from model farms and from control farms (control farms 1).

The second regression model (Regression Model 2), assesses the relationship between several factors that were only available for model farms and the risk of contamination at high level (≥1,000cfu/g in pooled caecal samples). The results of this model, presented in Table 6, show:

- Batches at depopulation had four times higher odds of colonisation than batches at thinning.
- Compared to the hybrid with highest risk of colonization (hybrid R), batches of Cobb hybrid had 46% of the odds (OR 0.46) of high colonisation and batches of Ross308 had 22 % of the odds of high colonization.
- Sheds that were kept empty for up to 1 week were less likely to produce highly colonized batches; they had half of the odds of high colonization than batches grown after a 1-2 week empty period.
- The odds of colonisation was 10.55 times higher in the summer of 2012 and 3.22 times higher in the summer of 2013 when compared with winter 2013 as a reference.

Table 6 Results of random effects logistic regression (Regression Model 2) investigating the contribution of selected factors in model farms to *Campylobacter* colonisation (defined as ≥1,000 cfu/g in pooled caecal samples). Results from a total of 1,135 batches sampled between 1st March 2012 to 31 August 2013[2] .

Factors	OR[i]	P-value [ii]	95% CI [iii]	
Harvest occasion				
Thinning	1.00	<0.001		
Depopulation	4.41		3.21	6.05
Type of hybrid				
Cobb	0.46	0.050	0.21	1.00
JA	1.51	0.669	0.23	10.04
R	1.00			
R308	0.22	<0.001	0.11	0.44
R708	0.46	0.143	0.16	1.30
Empty days				
up to 1 week	0.50	0.005	0.31	0.81
1 - 2 weeks	1.00			
2 – 3 weeks	0.83	0.504	0.48	1.44
> 3 weeks	1.85	0.233	0.67	5.08
Not available	5.77	0.013	1.46	22.82
Sampling period				
1 Mar - 31 May 2012	1.25	0.438	0.71	2.21
1 June – 31 Aug 2012	10.55	<0.001	5.27	21.10
1 Sept - 30 Nov 2012	0.92	0.726	0.57	1.49
1 Dec - 28 Feb 2013	1.00			
1 Mar - 31 May 2013	0.67	0.126	0.40	1.12
1 June - 31 Aug 2013	3.22	<0.001	1.79	5.77
Constant	1.88	0.069	0.95	3.70
standard deviation of random effects	0.81		0.47	1.38
Interclass correlation coefficient (rho)	0.17		0.06	0.37

[i] Odds ratio (OR) is a ratio of odds (a ratio of highly colonized batches vs. those that are less colonised <1000cfu/g) in a category of interest vs. odds in baseline category (i.e. OR is a ratio of other two ratios). The OR quantifies how strongly the presence of particular factor is associated with high colonisation in studied population.

[ii] The p-value is an indication of probability, with a value ranging from zero to one, of obtaining a test statistic result at least as large as the one that was actually observed, if there is no differences between compared groups.

[iii] The 95% confidence interval (95% CI) is an interval estimate of a parameter with 95% confidence. We are 95% confident that the true value of the parameter is in our confidence interval.

Likelihood-ratio statistic for comparison with a logistic model not adjusting for clustering (i.e. if rho=0): chibar2(01) = 27.76 Prob > chi2 <0.001

[2] Only batches sampled during this period included as it was the period of overlap between different factors recorded for batches from model farms

The results of comparing the odds of colonisation at depopulation for batches grown in model farms and in which thinning was carried out with batches, also from model farms, and in which thinning was not conducted are presented in table 7. In farms with enhanced biosecurity, flocks that are thinned have more than twice (2.34) the odds of colonisation at depopulation than flocks that are not thinned.

Table 7 Results of random effects logistic regression (Regression Model 3) investigating the effect of partial depopulation (thinning) on *Campylobacter* colonisation (defined as ≥1,000 cfu/g in pooled caecal samples) at depopulation. Results from a total of 1135 batches sampled between 1st March 2012 to 31 August 2013[3].

Factors	OR	p-value	95% CI	
The flock had not been partially depopulated	1.00	0.035		
The flock had been partially depopulated (thinned)	2.34		1.06	5.17
Sampling period				
1 Mar - 31 May 2012	3.61	0.001	1.74	7.50
1 June – 31 Aug 2012	11.72	<0.001	4.28	32.06
1 Sept - 30 Nov 2012	1.06	0.857	0.58	1.91
1 Dec - 28 Feb 2013	1.00			
1 Mar - 31 May 2013	1.78	0.069	0.96	3.33
1 June - 31 Aug 2013	1.95	0.036	1.04	3.64
Constant	1.23	0.665	0.48	3.19
standard deviation of random effects	0.75		0.44	1.28
Interclass correlation coefficient (rho)	0.15		0.06	0.33

Likelihood-ratio statistic for comparison with a logistic model not adjusting for clustering (i.e. if rho=0): chibar2(01) = 30.21 Prob > chi2 <0.001

[3] Only batches sampled during this period included as it was the period which overlaps with the period in Model 2

3.4. Population attributable fractions (PAF) in batches sent to slaughter

The detailed data associated with estimation of PAF are presented in Annexes B and C. Under the assumption that identified risk factors have a casual association with the colonisation of poultry batches, the following estimates were made:

- If all batches in the UK were raised under enhanced biosecurity an estimated 26% of colonized batches in the population would be avoided. This is under the assumption that no UK farms currently operate under enhanced biosecurity (with the exception of model farms in this study).
- If none of the batches were subject to thinning then an estimated 24% of colonized batches could be avoided. This value assumes that thinning is currently practised in 89% of batches (as observed in this study).
- If all batches were of the hybrid types of lower risk then between 17% and 33 % of batch colonisation can be prevented. In this study, 61% of batches were from those hybrids associated with higher risk of colonisation.
- Interventions against different factors could be introduced simultaneously. We estimate that approximately 30% of highly colonised batches would be avoided in a realistic scenario of successfully implementing biosecurity in half of the batches, avoiding thinning in a third of batches in which it is currently practiced and shifting to hybrids with lower risk of colonisation in half of the batches.

4. DISCUSSION

4.1. Key findings

This study analyses the impact of elevated biosecurity measures and selected husbandry factors on *Campylobacter* colonisation of broiler batches raised in UK farms from September 2011 to August 2013. The results of the analyses undertaken show with 95% confidence that elevated biosecurity was associated with decreased colonisation of batches at thinning, reducing the odds of high colonisation by 64% to 93%. At the time of depopulation, the effect of increased biosecurity is considerably lower. The point estimate still suggests that enhanced biosecurity is associated with a reduction in the risk of colonization at depopulation, although there is no enough statistical evidence to conclude with high confidence that batches raised under increased biosecurity have different probability of colonisation at depopulation than control batches raised under normal conditions. The strong association between enhanced biosecurity and colonization at time of thinning and the subsequent attenuation of this effect at time of total depopulation could indicate that enhanced biosecurity is more effective at delaying than preventing colonization.

There is evidence of an association between the number of empty days between flocks and colonisation: batches for which the shed had been kept empty less than a week appear to be at lower risk of colonisation than those for which the number of empty days was 8 - 14 or more; sheds for which information on empty days was not available appear to be at greater risk. Over two thirds of batches for which information was not available were slaughtered during the last three sampling (i.e. spring and summer) periods and originated from 4 farms.

The results strongly support the existence of an association between the type of hybrid and batch colonization.

As expected, the risk of colonisation exhibits a strong seasonality, with batches raised during winter at significantly lower risk of colonisation.

The flocks that had been partially depopulated (thinned) experienced a two times higher odds of colonisation at depopulation than batches which had not practised partial depopulation.

The results from testing pooled neck skin samples correlate well with the results from pooled caecal samples for batches that are negative in caeca, but only 40% of batches classified as highly colonized on the basis of caecal samples would be identified as highly colonized by neck skin testing.

It was estimated that one quarter of highly colonized batches could be prevented if all farms enhanced their biosecurity to similar standards of the model farms in this study. A similar effect could be achieved if none of the crops sent to the slaughterhouse had been subject to previous thinning. The potential effect of raising only hybrid types

identified to be of low risk was estimated to be between 17% and 33% reduction in the proportion of highly colonized batches.

4.2. Interpretation

The main finding of this study is that biosecurity has a protective effect on batch colonisation, which is evident at the time of thinning. However, this effect becomes much less apparent at the time of depopulation. In other words, increased biosecurity appears to delay colonisation but by the time of depopulation this protective effect has faded considerably. It is likely that thinning itself is at least in part responsible for the attenuation of the protective effect of biosecurity by the time of depopulation, as the role of thinning as a risk factor for infection has been well established (Ellis-Iversen et al., 2009; Newell et al., 2011) and is also identified in this study. The fact that thinning was applied to 89% of batches included in this study and the strong financial motivation of the practice suggest that ceasing it completely may not be feasible. The interaction of enhanced biosecurity with thinning vs. depopulation shows that the preventive effect of biosecurity fades after thinning, with batches raised under enhanced biosecurity being less likely to reach the time of thinning as heavily colonized but having a higher risk of infection post-thinning than batches raised under standard biosecurity. The differences in *Campylobacter* colonisation between the hybrids may be due to a biological characteristic of the birds, differences in the length of the cycle or unmeasured factors associated with the type of hybrid such as diet or specific husbandry practices. The effect of season on colonisation of batches has been extensively reported and tentatively attributed to the biological characteristics of *Campylobacter* spp. as well as seasonal changes in farm practices (Newell and Fearnley, 2003; Powell et al., 2012).

4.3. Limitations

A number of limitations of the study should be acknowledged. Although farms were recruited trying to avoid obvious departures from established poultry production practices, farm selection was not carried out probabilistically and selection bias as a result of systematic differences between the study farms and the general population of UK farms cannot be ruled out. Similarly, control farms were not selected probabilistically and differences with model farms, other than the level of biosecurity, cannot be ruled out. Lack of information on farm of origin for the main group of control batches prevented us from accounting for potential within-farm clustering and within-company clustering was considered instead. Despite these limitations, it seems unlikely that the main findings of the study are due to these potential biases.

4.4. Practical implications and future research

The situation of several European countries shows an overall high prevalence of *Campylobacter* in caeca of slaughtered poultry batches (EFSA, 2010a) and even higher figures have been reported for other continents (Kuana et al., 2008). A protective effect of on-farm hygiene interventions on batch colonisation is suggested in a UK review (Newell et al., 2011) and in epidemiologic research (Ellis-Iversen et al., 2012). This study provides empirical evidence of the potential of enhancing biosecurity as a means of reducing the proportion of heavily contaminated batches sent to slaughterhouses and eventually the proportion of heavily contaminated chickens on retail. The study also shows that the potential to mitigate the risk of heavily contaminated chicken reaching the consumer by enhancing biosecurity is limited and should therefore be combined with other measures further along the poultry chain. At the level of the farm, our study concurs with previous research identifying thinning as an important risk factor for colonization. The existence an interaction between enhanced biosecurity and thinning by which one modifies the effect of the other implies that potential interventions should consider both simultaneously. The association between breed and risk of colonization should be further explored as it is possible that factors other than the characteristics of the birds are responsible. A better understanding of this association could identify other aspects of the production system that could potentially be managed to mitigate the risk of colonization.

The results of this study justify the implementation of a small-scale intervention to confirm and quantify the impact of combined changes to biosecurity and thinning including monitoring beyond the abattoir. The study should ideally involve different breeds. Such an intervention could validate the findings of this study and allow more precise quantification of the likely public health benefits associated with the introduction of changes to biosecurity and thinning practices. Even though *Campylobacter* is referred to as the top pathogen associated with food borne disease in the EU there are no mandatory requirements for monitoring foodstuff on microbiological criteria as those contained in Commission Regulation (EC) No. 2073/2005 for other food-borne pathogens, including *Salmonella*. There are indications that the controls applied for *Salmonella* would not necessarily correlate with a decrease in the prevalence of *Campylobacter* spp. (Hue et al., 2010). Studies in the Netherlands (RIVM, 2013) and Nordic countries (Nauta, 2013) propose the implementation of threshold levels for batch colonisation at the end of slaughter. Further research along the lines of the intervention study proposed above could establish the basis for the implementation of threshold levels. The outcomes could support discussions at EU level on potential harmonised microbiological and other criteria for the production-processing chain.

5. CONCLUSIONS

- A substantial risk of *Campylobacter* spp. infection is present early in the food chain, as a large proportion of poultry batches (>60%) were colonized in caeca at high levels.
- The risk of batch colonisation exhibits seasonality, with a peak in summer when 100% of the studied batches were colonized.
- Enhancement of biosecurity in commercial poultry farms can contribute to the reduction of batch colonisation at thinning. Following thinning, the potential effect of increased biosecurity becomes much less apparent.
- Husbandry factors such as hybrid type and empty period between flocks were also associated with a decrease in batch colonisation at high levels ≥1,000cfu/g.
- It is likely that the detrimental impact of partial depopulation on batch colonisation minimizes the protective role of biosecurity and other protective 'on-farm' factors at the end of production cycle.

ACKNOWLEDGMENTS

We would like to express our gratitude to the Food Standards Agency and other members of Joint Working Group on *Campylobacter*. We wish also to thank the companies who initiated and implemented the project for the provision of raw data, clarifications with regard to data available and advise and support along the project, to Rebecca Pereira for the initial descriptive statistics on a subset of data available until January 2013, to Dr Joey Ellis-Iversen for comments and advise and to all those who were involved in logistics, sample collection and laboratory analyses.

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ANNEX A

Table 8 The training, cleaning, water and feed pre-requisites for all farms involved in the project with a description of the requirements, the staff that need to complete these requirements, the date by which these requirements should be completed/started and any necessary documentation that should be included (Pereira 2013).

Activities	Description	Staff	Date	Documentation
Training	Compliance with the new Assured Chicken Production (ACP) biosecurity standards	All farm level staff	Completed by 1 October 2011	ACP report from each farm once a year
	Understanding the risks of *Campylobacter* and how to protect public health	Area manager Farm managers Farm staff Catcher managers Model farm catchers	Completed by April 2011	Staff training via a presentation and Q/A session Online questionnaire as refresher training
		Farm managers		Report for each flock noting incidents of non-compliance
	Introduction of Hazard Analysis and Critical Control Points (HACCP) and compliance with these controls		Starting from 1 October 2011	
Cleaning	Compliance with cleaning schedules	Farm manager	Two audits per year	Testing for *Campylobacter* in routine sampling for sheds

Activities	Description	Staff	Date	Documentation
Water	Usage of potable water with/without additives	Farm manager	Starting from 1 August 2011	Record of dates and any additives used (if applicable)
	Any additional water treatments to be monitored	Farm manager	Starting from 1 August 2011	Record of stop dates, start dates, crop number and sheds with reduced *Campylobacter* counts
	No lifting of water less than one hour before catching	Farm manager	Starting from 1 August 2011	Record of stop dates, start dates and crop number
Feed	Any food additives used that will affect *Campylobacter* must be recorded, other additives to be kept confidential	Farm manager	Starting from 1 August 2011	Record of dates and dosage
	No lifting of feed less than six hours before catching	Farm manager	Starting from 1 August 2011	Record of the length of time between when the feed was lifted and when it was replaced

Table 9 The biosecurity interventions for all model farms involved in the project with a description of the requirements, the staff that need to complete these requirements, the date by which these requirements should be completed/started and any necessary documentation that should be included (Pereira 2013).

Level of biosecurity	Description	Staff	Date	Documentation
Standard Biosecurity	ACP standards in place Compliance with the Red Tractor assurance scheme	Farm manager	Starting from April 2011	Annual ACP audit Reports of any exceptional cases
Additional Biosecurity	Treat each shed as a bio-secure unit with all protective clothing and equipment being shed specific	Agricultural managers	Starting from 1 August 2011	Farm manager report
Thinning	All catchers briefed on the additional biosecurity protocol	Catcher manager		Catcher team leader report
Transport	Thinning transportation should be clean and dry Transportation for thinning should be used first in the day	Transport manager Transport manager		Farm manager report classed as: A – clean and dry B – clean but wet C – Dirty Records of transport vehicles

Level of biosecurity	Description	Staff	Date	Documentation
Depopulation	Aim to depopulate within five days of thin to reduce *Campylobacter* load if introduced at thinning	Planning team/ Agricultural managers		Records of the number of days between thin and depopulation for every shed
Shed Rest	Maximum period of shed rest while still meeting commercial needs	Agricultural managers		Records of the number of days all the sheds are empty

Table 10 Stratified analysis of potential risk factors for *Campylobacter* contaminated batches (≥1,000 cfu/g).

Variable	Time	Exposure	Number (%) of Batches >1,000cfu/g	Number of Batches <1,000cfu/g	p-value (chi2)
Harvest occasion					
Model Farms (MF)	1-16	Thinning	438 (54.5)	365	**<0.001**[i]
		Depopulation	670 (75.9)	213	
		at D when (T+)	614 (77.9)	174	**<0.001**
		at D when (T-)	56 (58.9)	39	
Control farms 1 (C1)	6-17	Thinning	177 (88.9)	22	0.888
		Depopulation	177 (88.5)	23	
Control farms 2 (C2)	12-16	Thinning	12 (75.0)	4	0.336 [F]
		Depopulation	13 (92.9)	1	
Biosecurity					
at thinning	6-16	Model Farms	367 (58.3)	262	**<0.001**
		Controls 1	163 (89.1)	20	
at depopulation	6-16	Model Farms	517 (79.1)	137	**0.002**
		Controls 1	163 (89.1)	20	
at thinning	12-16	Model Farms	141 (48.5)	150	**<0.001** (MF vs C1)
		Controls 1	70 (86.4)	11	**0.043** [F] (MF vs C2)
		Controls 2	12 (75.0)	4	0.264 [F] (C1 vs C2)
	12-16	Model Farms F,G,H,I,J	55 (56.1)	43	0.182 [F] (MF vs C2)
at depopulation	12-16	Model Farms	221 (75.2)	73	**0.004** (MF vs C1)
		Controls 1	72 (90.0)	8	0.201 [F] (MF vs C2)
		Controls 2	13 (92.9)	1	1 [F] (C1 vs C2)
	12-16	Model Farms F,G,H,I,J	83 (88.3)	11	1 [F] (MF vs C2)
Welfare					
at thinning in model farms		Enhanced	225 (60.0)	150	**0.003**
		Standard	195 (49.4)	200	
at depopulation in model farms		Enhanced	335 (80.1)	83	**0.002**
		Standard	307 (71.4)	125	
Hybrid type					
at thinning in model farms		Cobb (C)	56 (51.4)	53	0.096 among groups*
		R	188 (65.5)	99	
		C & R	7 (100.0)	0	
		Ross 308	45 (47.4)	50	**<0.002** [F] (Ross308

Variable	Time	Exposure	Number (%) of Batches >1,000cfu/g	Number of Batches <1,000cfu/g	p-value (chi2)
		Ross 708	26 (60.5)	17	vs J) **<0.002** (Ross308 vs R)
		JA (J)	18 (85.7)	3	
		na[ii]	97 (40.4)	143	**<0.001** among groups& na
at depopulation in model farms		Cobb	103 (84.4)	19	0.346 among groups **<0.009** [F] (Ross308 vs J) 0.171 [F] (Ross308 vs C&R) **<0.004** among groups& na
		R	248 (89.5)	29	
		C & R	9 (90.0)	1	
		Ross 308	81 (66.9)	40	
		Ross 708	32 (80.0)	8	
		JA	19 (95.0)	1	
		na	177 (60.6)	115	
Empty days					
at thinning in model farms		1-7days	86 (56.6)	66	0.093 among groups
		8-14 days	253 (55.6)	202	
		15-21 days	23 (42.6)	31	
		22-47 days	15 (0.75)	5	
		na	61 (50.0)	61	
at depopulation in model farms		1-7days	144 (77.4)	42	0.152 among groups
		8-14 days	367 (76.8)	111	
		15-21 days	43 (76.89)	13	
		22-47 days	22 (88.9)	3	
		na	94 (68.1)	44	
Days to depopulation (from thinning to depopulation)					
at thinning in model farms		0-2 days	28 (63.6)	16	**0.032** among groups **<0.001** (3-4 vs 12-14) 0.093 (3-4 vs 0-2) 0.054 among groups& na
		3-4 days	82 (49.4)	84	
		5-6 days	128 (59.9)	114	
		7-11 days	102 (50.2)	101	
		12-14 days	46 (85.2)	8	
		15-21 days	7 (53.8)	6	
		na	45 (55.6)	36	
at depopulation		0-2 days	36 (80.0)	9	0.289 among

Variable	Time	Exposure	Number (%) of Batches >1,000cfu/g	Number of Batches <1,000cfu/g	p-value (chi2)
in model farms		3-4 days	109 (65.7)	57	groups
		5-6 days	193 (77.8)	55	
		7-11 days	173 (84.8)	31	**0.004** (3-4 vs 12-14)
		12-14 days	45 (86.5)	7	**<0.001** (3-4 vs 7-11)
		15-21 days	15 (100.0)	0	
		na	99 (64.7)	54	0.162 among groups& na
Companies					
at thinning in control farms 1		Q	61 (87.1)	9	0.962 among groups
		R - not tested	na	na	
		S	55 (91.7)	5	
		T	61 (88.4)	8	
at depopulation in control farms 1		Q - not tested	na	na	0.887 among groups
		R	66 (90.4)	7	
		S	51 (83.6)	10	
		T	60 (90.9)	6	

[F] Fisher's exact test was used because of the small number (≤5) in the outcomes

* Chi2 test, comparing observed and expected frequencies, excluding group with zero values; further two comparisons were conducted between the group with lowest value and two groups with highest non 100 percent values

[I] p-values are indicated **in bold** when they are significant (<0.05)

[ii]na: information not available

[per] percentile in distribution of data

ANNEX B Statistical analysis – technical details

Univariate statistics

All factors in the dataset were analyzed individually as exposure variables potentially contributing to a high colonisation of batches. Two hypotheses were tested: ($H_0$1) that batches grown in enhanced biosecurity have the same odds of being highly colonized with *Campylobacter* as batches originating from normal biosecurity farms and ($H_0$2) that batches characterised by the presence of an 'on-farm' factor experienced the same odds of high colonisation as batches without that factor. The level of statistical evidence against hypothesis (H_0) was checked through X^2 tests. P-values <0.05 were considered as significant. Further stratification was applied to control for the effect of factors influencing the outcome simultaneously.

An additional investigation on the impact of enhanced biosecurity was conducted in a comparison of batches from matched farms in model farms and control farms 2. The five control farms were studied between January 2013 and August 2013. The concordant and discordant pairs of farms were recorded at levels ≥1,000cfu/g and <1,000cfu/g. McNemar's test with the continuity correction and binomial test (Altman et al., 2000) were used. The reliability of data was assessed by investigating the total number of discordant pairs.

Multivariate analyses

Further statistical modeling was used to quantify the role of biosecurity and multiple farm factors on batch colonisation. Three random effects logistic regression models (Regression Model 1, Regression Model 2 and Regression Model 3) were developed. The study periods cover the time from 16 April 2012 to 31 August 2013 (Model1) and from 1 March 2012 to 31 August 2013 (Model2 and Model 3).

Exposure variables with p-value <0.10 from univariate analyses were included in the initial modeling. Variables with p-value >0.10 in the multivariate model were excluded one by one starting from the highest p-values. Factors were grouped into two or more categories based on available dichotomous data for biosecurity, harvest occasion (thinning or depopulation) and welfare, and the records per sampling period (). Hybrid types, empty days and days to depopulation were categorized – the latter two variables based on percentiles. The explanatory power of the models and potential interactions were checked using the likelihood ratio statistic (LRS) comparing a more complex with the simpler model. Models which assumed a linear relationship between potential risk factors and batch colonisation were compared with models that allowed for non-linear relationships. When estimating odds ratios, the category with the lowest odds was selected for the baseline.

Farms were accounted for as a cluster variable (Level 2 variable) for batches (Level 1 variable) nested in the farms. A more complex model with three level data was attempted in and rejected as the inclusion of another nested variable (shed) into the farms indicated very low standard deviation <0.001 at that level. The random effect was applied to three models to account for clustering at farm level as batches are more similar in the farm rather than with batches from other farms. The value of interclass correlation was investigated to understand the proportion of variation in the model attributed to farms. The accuracy of the models was checked by increase in the integration points of the quadrature approximation and observing whether the obtained relative differences present considerably low (<0.01) values. The harvest occasion (thinning or depopulation) and sampling period were included in Model 1 as the only other common factors in model farms and control farms 1.The potential confounding of time in the year when samples are taken is controlled in the model and the option for interaction tested by LRS and considered as biologically plausible between harvest occasion and biosecurity. The post-estimation of odds ratio (OR) and confidence interval 95% C.I. were estimated for each group affected by interaction.

The sampling periods together with all 'on farm' factors available for model farms were included in Model 2 as they exhibited p-values <0.050 in the univariate analysis. Welfare and days to depopulation did not show evidence of association with the odds of High levels of colonisation in the model (p-value >0.200). The category of hybrids indicated as C&R was excluded because of collinearity with depopulation on the predicted outcome of the model and the small number of batches (17).

In Model 3, we have investigated the effect of pre-harvest practice (thinning) on *Campylobacter* colonisation of batches in model farms, adjusting for the season. We have compared the results at depopulation of 45 batches were thinning was not practised with 656 batches with applied thinning during the period investigated in Model 2 from 1 March 2012 to 31 August 2013.

Population attributable fraction (PAF)

Initially, we estimate the PAF from Model results through a statistical package *punafcc* in STATA12. However, those values are obtained when a hypothetical scenario of successfully elimination of the effect of a factor is achieved in all batches (assuming that the association between a factor and high colonisation is casual) and that scenario is compared to observed data in the study. Further, a mathematical approach was followed to explore different scenarios of exposure to factors and expected PAF.

The OR obtained from modelling were converted to adjusted relative risk (RRa) values (Zhang and Yu, 1998) and used to estimate population attributable fraction (PAF) (Potter et al., 2010). The values of population attributable fractions (PAF) are estimated as the prevalence of exposure among cases (i.e. highly colonised batches) is

taken into account rather than prevalence of the exposure to the factors only (Williamson, 2010).

$$[Pd*(RRa-1)]/RRa = PAF \qquad (eq.1)$$

However, the decisions for intervention would be linked more directly to the prevalence of the exposure to factors. A mathematical expression of the relation between prevalence of the exposure to factors and levels of exposure among cases was explored in the following equations:

$$Pe_{ij} * R_{ij} = D_{ij} \qquad (eq.2)$$

$$D_{ij} /(D_{ij} + D_{ij+1}) = Pd_{ij} \qquad (eq.3)$$

$$(Pe_{ij} * R_{ij})/[R_{ij+1} + (Pe_{ij} * R_{ij}) - R_{ij+1}] = Pd_{ij} \qquad (eq.4)$$

Pe_{ij}: prevalence of the exposure to factor i in category j
R_{ij}: risk to factor i in category j
D_{ij}: cases due to factor i in category j
Pd_{ij}: prevalence of the exposure to factor i in category j

Several hypothetical values of prevalence of the exposure to factors are explored to present different options for PAF in 'real world' situations.

Combination of PAF (PAFc) is possible on a multiplicative scale as the factors are considered in the modelling in a multiplicative way:

$$PAFc= 1 – [(1-PAF1)*(1-PAF2)….*(1-PAFn)] \qquad (eq.4)$$

ANNEX C Scenarios of PAF considering various level of exposure

Table 11 Adjusted relative risks (RRa) estimated from the results of Model 1 and observed risk in the categories of factors

Factors	RRa*	Risk %	RRa 95% CI	
Biosecurity				
Control farms1	1.00	89.1		
Model farms	0.64	68.9	0.41	0.84
Harvest occasion				
Thinning (T)	1.00	65.3		
Depopulation (D)	0.96	81.2	0.68	1.20
Interaction between biosecurity & harvest occasion				
Effect of standard biosecurity**				
- at thinning	1.35	68.9	1.25	1.41
- at depopulation	1.15	68.9	0.92	1.30
Effect of Depopulation:				
- in model farm	1.31	65.3	1.25	1.36
- in control farms1	0.96	65.3	0.68	1.20
Sampling period				
16 Apr – 31 May 2012	1.44	85.0	.31	1.53
1 June – 31 Aug 2012	1.56	92.6	1.50	1.61
1 Sept - 30 Nov 2012	1.10	66.6	0.95	1.23
1 Dec - 28 Feb 2013	1.00	59.8		
1 Mar - 31 May 2013	0.99	60.3	0.84	1.13
1 June - 31 Aug 2013	1.39	81.8	1.29	1.49

*OR converted in RRa via the following formula RRa = OR/[(1-Risk at baseline)+(Risk at baseline*OR)] (Zhang and Yu, 1998)

**OR in model farms is converted to OR in control farms (OR 6.25 at thinning and OR 1.75 at depopulation) and then converted to RRa

Various proportions of exposure to factors and estimated mathematically adjusted PAF from Model 1 are shown in Table 13 to allow inferences for different situations. For example: In case of successfully implementation of enhanced biosecurity in nearly all control farms (0.95%) we can expect approximately 25% reduction of highly colonised batches achieved mainly in batches at thinning. If only 70% of control farms change to the standards of model farms the reduction can be 1/5 and If only 50 % of all farms applied enhanced biosecurity we can still expect a positive effect in 1 out of 7 colonised batches. The impact of depopulation is similar in the numerical values although it may not be feasible to remove that factor. Several PAF were estimated mathematically from the results of Model 2. Results are presented at different exposure scenarios in Table 16. The obtained PAF from the result of Model 3 are presented in Table 17.

Table 12 The exposure of batches in model and control farms during the period 16 Apr 2012 – 31 Aug 2013

Factor	exposure	Cases	Risk %	Prevalence of exposure (Pe)	Prevalence of exposure in cases (Pd)
Biosecurity					
Control farms	366	326	89.1	0.22	0.27
Model farms	1283	884	68.9	0.78	0.73
Harvest					
Batches at thinning	812	530	65.3	0.49	0.44
Batches at depopulation	837	680	81.2	0.51	0.56
Sampling period					
16 Apr – 31 May 2012	160	136	85.0	0.10	0.11
1 June – 31 Aug 2012	299	277	92.6	0.18	0.23
1 Sept - 30 Nov 2012	308	205	66.6	0.19	0.17
1 Dec - 28 Feb 2013	301	180	59.8	0.18	0.15
1 Mar - 31 May 2013	295	178	60.3	0.18	0.15
1 June - 31 Aug 2013	286	234	81.8	0.17	0.19

Table 13 Population attributable fractions (PAF) estimated from Model 1per several levels (0.05 to 1.00) of prevalence of the exposure

Hypothetical prevalence of the exposure (Pe) in batches (i.e. proportion of batches exposed to a factor)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.95	1.00
Effect of biosecurity												
PAF[i] in batches at thinning	**0.02**	**0.03**	**0.06**	**0.09**	**0.12**	**0.15**	**0.17**	**0.20**	**0.22**	**0.24**	**0.25**	**0.26**
Estimated proportion of highly colonised batches exposed to (Pd) [ii] control farm	0.06	0.13	0.24	0.36	0.46	0.56	0.66	0.75	0.84	0.92	0.96	1.00
PAF in batches at depopulation	0.00[iii]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Effect of depopulation												
PAF in model farms	**0.01**	**0.03**	**0.06**	**0.08**	**0.11**	**0.13**	**0.15**	**0.18**	**0.20**	**0.22**	**0.23**	**0.24**
Estimated proportion of highly colonised batches exposed to depopulation	0.06	0.12	0.24	0.35	0.45	0.55	0.65	0.74	0.83	0.92	0.96	1.00
PAF in control farms	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sampling period												
PAF 16 Apr – 31 May 2012	**0.02**	**0.04**	**0.08**	**0.12**	**0.15**	**0.18**	**0.21**	**0.23**	**0.26**	**0.28**	**0.29**	**0.30**
Estimated proportion of highly colonised batches of exposed to the sampling period above	0.07	0.14	0.26	0.38	0.49	0.59	0.68	0.77	0.85	0.93	0.96	1.00
PAF 1 June – 31 Aug 2012	**0.03**	**0.05**	**0.10**	**0.14**	**0.18**	**0.22**	**0.25**	**0.28**	**0.31**	**0.34**	**0.35**	**0.36**
Estimated proportion of highly colonised batches of exposed to the sampling period above	0.08	0.15	0.28	0.40	0.51	0.61	0.70	0.78	0.86	0.93	0.97	1.00
PAF 1 Sept - 30 Nov 2012	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PAF 1 Dec - 28 Feb 2013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Hypothetical prevalence of the exposure (Pe) in batches (i.e. proportion of batches exposed to a factor)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.95	1.00
PAF 1 Mar - 31 May 2013	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PAF 1 June - 31 Aug 2013	**0.02**	**0.04**	**0.07**	**0.10**	**0.14**	**0.16**	**0.19**	**0.22**	**0.24**	**0.26**	**0.27**	**0.28**
Estimated proportion of highly colonised batches of exposed to the sampling period above	0.07	0.13	0.25	0.37	0.48	0.58	0.67	0.76	0.85	0.92	0.96	1.00

[i] PAF estimated by the following formula PAF=Pd*(RRa-1)/RRa (Williamson, 2010) as described in in (*eq. 1*) Annex B

[ii] estimation takes into account Table 11and using the formula (Peij *Rij)/[Rij+1 + (Peij * Rij) - Rij+1] = Pdij) as described in (*eq. 4*) Annex B

[iii] zero value is estimated for PAF when CI95% of RRa includes zero

Table 14 Adjusted relative risks (RRa) estimated from the results of Model 2 and observed risk in the categories of factors

Factors	RRa*	Risk %	RRa 95% CI	
Harvest occasion				
Thinning (T)	1	60.0		
Depopulation (D)	1.45	83.3	1.38	1.50
Type of hybrid [i]				
Cobb	1	68.8		
R compared [ii] with Cobb	1.20	77.3	1.00	1.33
R 308	1	58.3		
R compared with R308	1.48	77.3	1.30	1.59
Empty days				
up to 1 week	1	0.73		
1 - 2 weeks compared with 'up to 1 week'	1.16	71.2	1.05	1.23
na[iii] compared with 'up to 1 week'	1.33	94.0	1.29	1.38
Sampling period				
1 June – 31 Aug 2012	1.58	94.1	1.49	1.63
1 Dec - 28 Feb 2013	1	59.3		
1 June - 31 Aug 2013	1.39	81.4	1.22	1.51

[i] RRa were not calculated for hybrids which results of model 2 includes zero values in $CI_{95\%}$

[ii] OR in Cobb and R308 were converted to OR in hybrid R and then converted to RRa; OR converted in RRa via the following formula RRa = OR/[(1-Risk at baseline)+(Risk at baseline*OR)] (Zhang and Yu, 1998)

[iii] na: information is not available

Table 15 Adjusted relative risks (RRa) estimated from the results of Model 3 and observed risk in the categories of factors

Factors	RRa*	Risk %	RRa 95% CI	
Thinning **not** practised	1	64.4		
Thinning practised	1.13	80.5	1.01	1.19

Table 16 Population attributable fractions (PAF) estimated from Model 2 per several levels (0.05 to 1.00) of prevalence of the exposure

Hypothetical prevalence of the exposure (Pe) in batches (i.e. proportion of batches exposed to a factor)	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**0.95**	**1.00**
Effect of depopulation												
PAF[i]	**0.02**	**0.04**	**0.08**	**0.12**	**0.15**	**0.18**	**0.21**	**0.24**	**0.26**	**0.29**	**0.30**	**0.31**
Estimated proportion [ii] of highly colonised batches exposed to depopulation	0.07	0.13	0.26	0.37	0.48	0.58	0.68	0.76	0.85	0.93	0.96	1.00
Type of hybrid												
PAF in hybrid R if compared with Cobb	**0.01**	**0.02**	**0.04**	**0.05**	**0.07**	**0.09**	**0.11**	**0.12**	**0.14**	**0.15**	**0.16**	**0.17**
Estimated proportion of highly colonised batches if compared with Cobb	0.06	0.11	0.22	0.33	0.43	0.53	0.63	0.72	0.82	0.91	0.96	1.00
PAF in hybrid R if compared with R308	**0.02**	**0.04**	**0.08**	**0.12**	**0.15**	**0.19**	**0.22**	**0.25**	**0.27**	**0.30**	**0.31**	**0.33**
Estimated proportion of highly colonised batches if compared with R308	0.07	0.13	0.25	0.36	0.47	0.57	0.67	0.76	0.84	0.92	0.96	1.00
Empty days												

Hypothetical prevalence of the exposure (Pe) in batches (i.e. proportion of batches exposed to a factor)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.95	1.00
PAF in 1 - 2 weeks compared with 'up to 1 week'	**0.01**	**0.01**	**0.03**	**0.04**	**0.05**	**0.07**	**0.08**	**0.10**	**0.11**	**0.12**	**0.13**	**0.14**
Estimated proportion of highly colonised batches in 1 – 2 weeks if compared with 'up to 1 week'	0.05	0.10	0.20	0.31	0.41	0.51	0.61	0.71	0.80	0.90	0.95	1.00
PAF if information is not available compared with 'up to 1 week'	**0.02**	**0.03**	**0.06**	**0.09**	**0.12**	**0.14**	**0.16**	**0.19**	**0.21**	**0.23**	**0.24**	**0.24**
Estimated proportion of highly colonised batches in 1 - 2 weeks if compared with 'up to 1 week'	0.06	0.13	0.25	0.36	0.47	0.57	0.66	0.75	0.84	0.92	0.96	1.00
Sampling period												
PAF 1 June – 31 Aug 2012	**0.03**	**0.06**	**0.10**	**0.15**	**0.19**	**0.23**	**0.26**	**0.29**	**0.32**	**0.34**	**0.36**	**0.37**
Estimated proportion of highly colonised batches of exposed to the sampling period above	0.08	0.15	0.28	0.40	0.51	0.61	0.70	0.79	0.86	0.93	0.97	1.00
PAF 1 June - 31 Aug 2013	**0.02**	**0.04**	**0.07**	**0.10**	**0.13**	**0.16**	**0.19**	**0.21**	**0.24**	**0.26**	**0.27**	**0.28**
Estimated proportion of highly colonised batches of exposed to the sampling period above	0.07	0.13	0.26	0.37	0.48	0.58	0.67	0.76	0.85	0.93	0.96	1.00

[i] PAF estimated by the following formula PAF=Pd*(RRa-1)/RRa (Williamson, 2010); as described in in (*eq. 1*) Annex B; PAF is considered to be zero when categories of factors include zero values in $CI_{95\%}$ of results in Model 2.

[ii] estimation takes into account Table 14 and using the formula (Peij *Rij)/[Rij+1 + (Peij * Rij) - Rij+1] = Pdij) as described in (*eq. 4*) Annex B

Table 17 Population attributable fractions (PAF) estimated from Model 3 per several levels (0.05 to 1.00) of prevalence of the exposure

Hypothetical prevalence of the exposure (Pe) in batches (i.e. proportion of batches exposed to a factor)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.95	1.00
Effect of depopulation												
PAF [i]	**0.01**	**0.01**	**0.03**	**0.04**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**0.10**	**0.11**	**0.11**
Estimated proportion of highly colonised batches exposed to depopulation [ii]	0.06	0.12	0.24	0.35	0.45	0.56	0.65	0.74	0.83	0.92	0.96	1.00

[i] PAF estimated by the following formula PAF=Pd*(RRa-1)/RRa (Williamson, 2010); as described in in (*eq. 1*) Annex B; PAF is considered to be zero when categories of factors include zero values in $CI_{95\%}$ of results in Model 2.

[ii] estimation takes into account Table 15 and using the formula (Peij *Rij)/[Rij+1 + (Peij * Rij) - Rij+1] = Pdij) as described in (*eq. 4*) Annex B