Effect of enhanced biosecurity and selected on-farm factors on campylobacter colonization of chicken broilers

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Running head: Effect of biosecurity on campylobacter

SUMMARY

Human campylobacteriosis is the most commonly reported gastrointestinal bacterial infection in the EU; poultry meat has been identified as the main source of infection. We tested the hypothesis that enhanced biosecurity and other factors such as welfare status, breed, the practice of partial depopulation and number of empty days between flocks may prevent *Campylobacter* spp. caecal colonization of poultry batches at high levels (above 123000 cfu/g in pooled caecal samples). We analyzed data from 2314 poultry batches sampled at slaughter in the UK in 2011-2013. We employed random effects logistic regression to account for clustering of batches within farms and adjust for confounding. We estimated population attributable fractions using adjusted risk ratios. Enhanced biosecurity reduced the odds of colonization at partial depopulation (OR 0.25; 95%C.I. 0.14-0.47) and, to a lesser extent, at final depopulation (OR 0.47; 95%C.I. 0.25-0.89). An effect of the type of breed was also found. Under our assumptions, approximately 1/3 of highly colonized batches would be avoided if they were all raised under enhanced biosecurity or without partial depopulation. The results of the study indicate that on-farm measures can play an important role in reducing colonization of broiler chickens with *Campylobacter* spp. and as a result human exposure.

INTRODUCTION

Campylobacter spp. are the most commonly reported gastrointestinal bacterial pathogen in humans in the EU, responsible for an estimated cost of EUR 2.4 billion a year [1, 2].

Campylobacter jejuni is the species most frequently identified in human cases. The course of disease varies in severity from three to six days of diarrhoea to development of complications, including pancreatitis, arthritis and neurological disorders [3]. Poultry meat is considered the main source of human campylobacteriosis [4], and the intestines of commercial broilers (*Gallus gallus*) are often colonized [5, 6]. Microbial genetic data has provided further evidence of linkages between *Campylobacter* spp. strains in poultry and humans [7, 8]. 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% of all human cases of *Campylobacter jejuni* to the chicken reservoir as a whole[9]. An EFSA survey across 26 EU countries and two other countries in Europe in 2008 [10] showed an average of 71.2 % and ranged from a minimum of 2.0% to a maximum of 100.0% poultry batches testing positive at slaughter.

The pathogen may be introduced from the environment [11, 12] to poultry houses via different routes including houseflies [13], farmers' boots during daily operations or staff during partial depopulation [14]. Further horizontal transmission occurs from infected individuals to the surrounding environment and to other susceptible birds [15]. and colonization (presence of *Campylobacter* spp. in birds' intestine) of the entire flock occurs within a matter of a few days [16]. Theoretically, enhanced biosecurity in commercial farms could reduce the risk of batch colonization. However, there is limited empirical evidence that supports this hypothesis. As shown by an extensive literature review on the subject [15], study results are often questionable due to differences in implementation and poor study design and analysis. Besides the enhancement of biosecurity, several 'on farm' strategies have been proposed to reduce the risk of flock colonization and spread including chlorinated drinking water [17], bacteriophage therapy [18] and bacteriocins [19] or the use of probiotics [20] and vaccination [21]. However, many of those are still currently in development or considered not feasible. Evidence to assess the rationale of implementing feasible on-farm interventions such as enhancement of biosecurity is therefore urgently needed.

Between September 2011 and August 2013, the UK poultry industry implemented a plan 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'. We present an analysis of these data, including comparison of the levels of campylobacter caecal colonization in batches raised in 'model farms' under enhanced biosecurity with control batches from farms with 'standard biosecurity'.

MATERIALS AND METHODS

Study population and data sources

We investigated campylobacter colonization in broiler chickens slaughtered in the UK between 1 September 2011 and 31 August 2013.

Selection of 'model' farms

Sixteen farms were selected by the industry as 'model' examples, where a new protocol for enhanced biosecurity was implemented from August 2011. Although no formal probabilistic selection of candidate farms for enhancement of biosecurity was conducted, the 16 farms (denoted with alphabetic characters from A to O) were considered to apply standard production practices as in other broiler farms in the UK, were geographically dispersed and belonged to three different companies. Farm staff were trained and operated each poultry house (shed) as a bio-secure unit using dedicated tools, garments and footwear, protective clothes and shed-specific equipment, including for garbage and collection of dead birds, in addition to implementing standard procedures and highlighting the importance of having specific entry and exit procedures with washing and disinfection facilities for each poultry house. After the project, the procedures of enhanced biosecurity were shared with all farmers and a visual guide was prepared by FSA and National Farmers Union (NFU) http://www.nfuonline.com/fsa-infographic-campylobacter-biosecurity-cmyk-v3-lh-250615_not-signed-o/

Some more details on applied biosecurity measures in model farms are available in Table S1 and Table S2 in the *Supplementary Material* (available on the Cambridge Journals Online website). Model farms were located in England, Wales, Scotland and Northern Ireland and linked to different retailers. The number of sheds ranged from 1 to 12 per farm.

Selection of 'model' batches

Batches of chickens (birds which had been grown in the same shed and delivered to a slaughterhouse on one single day) were the study unit. Data were collected for 1,749 batches from model farms. Batches were selected so that all sheds would be sampled during the study. For purpose of data analysis, the 2-year study period was divided into 16 intervals of 45 days and each batch allocated to one of the 16 intervals based on the date when it was sent to the slaughterhouse.

Selection of control farms and batches

Three groups of control batches were investigated, as follows:

Broilers originated from different farms where standard biosecurity was applied (i.e. compliance with the Red Tractor assurance scheme http://assurance.redtractor.org.uk/).

1. “control batches 1” were selected in four poultry processing plants. . Information on the number of farms and origin of the batches was not available for analysis. Between April 2012 and October 2013, 366 batches were selected based on subjective assessments by the company veterinarians as batches of similar age, kept under similar conditions and slaughtered in the same week as the batches from farms with enhanced biosecurity.

2. “control batches 2” originated from five farms selected to match five of the model farms for all factors except biosecurity. A total of 30 batches were selected from these farms matched by week of slaughter to the corresponding ‘model’ batches.

3.”control batches 3” originated from 5 farms selected to match 5 model farms (A, B, C, D and E) for all factors with the exception of biosecurity. Information was collected for 136 batches in this group. Chickens were tested at thinning (partial depopulation) and also at final depopulation. We did not combine the batches from control farms 3 with those in control farms 2 as the investigation period was different.

Sample collection and laboratory testing

For each of the study batches, samples were taken from the caeca of five birds in the batch in the beginning of slaughter at the time of evisceration and pooled as a single sample. Samples were also taken from neck skins of three birds in the batch immediately after chilling at the end of slaughter line and pooled as a single sample. The birds’ carcasses were selected in a non-systematic way. All samples were tested to enumerate *Campylobacter* spp. without further speciation according to the agreed standards of International Organization for Standardization (ISO) ISO10272-2 2006. The

methodology was considered to be well established and was harmonized between the laboratories used by the three poultry companies involved in the study. Results therefore allow comparison between levels in caeca and neck skin and, further, with data from ongoing national monitoring in slaughterhouses in the UK.

Batch-level Risk Factors

For batches grown in 'model farms', information was obtained on other husbandry factors which could potentially have an influence upon colonization of broilers, namely:

Welfare status (data available for all 1,749 batches), defined as:

- 'Higher': broilers can be reared in the flock up to 30 kg/m^2, with added enrichments [play-bales, perches and artificial play-objects), the glass area of the windows is a minimum of 1-3 % of the floor area, according to 'Red Tractor' standards; or
- 'Freedom Food': stocking density is up to 30 kg/m^2 and rearing of a slow growing hybrid (JA 87) is required;, or
- 'Standard': maximum stocking density is over 30 kg/m^2.

Number of empty days between flocks (available for 1,693 batches, 96.8%).

Number of days from partial depopulation (thinning) to the end of the production cycle (available for 1,568 batches of the 1,654 batches where thinning was practiced, 94.8%).

Type of broiler hybrid (available for 1,745 batches, 99.8%).

Data analysis

In our analysis the outcome was a binary variable: based on caeca results, batches were classified as highly colonized vs. not highly colonized. To classify a batch as 'highly colonized' based on caeca results we used the threshold value that corresponds with a neck skin count above 3 $\log_{10}$, which is used as the high-risk threshold related to public health, jointly accepted by FSA and the poultry industry. The derivation of this value was as follows.

Defining a threshold for high levels of campylobacter colonization

We examined the frequency distributions of the counts of *Campylobacter* spp. in caeca and neck skin samples at different percentiles (1th, 5th, 10th, 25th, 35th, 50th, 65th, 75th, 90th, 95th, 99th, and the maximum values). In each of the specified percentiles, we calculated the difference between results

in caeca and neck skin using a $\log_{10}$ scale. The 95% C.I. for the resulting distribution of these differences was obtained. The value at the lower confidence limit for this difference was added to the level of 3 $\log_{10}$ of neck skin colonization. This was done because of the interest in defining a high-risk threshold based on caeca results.

Identification of factors associated with high levels of campylobacter colonization

The risk of being a highly colonized batch was estimated for: batches raised under enhanced biosecurity vs. batches raised under standard biosecurity (controls); batches harvested at thinning (partial depopulation) vs. at the end of the cycle (depopulation); batches composed of different hybrids: (Cobb 500, Cobb 500& Ross 308, Ross 308, Ross 708 and JA 87); batches with different empty days before the start of the cycle: (1-7, 8-14, 15-21 and 22-47); batches with different number of days between thinning and depopulation: (1-3, 4-6, 7-9, 10-12 and 13-18); batches for which welfare was ‘standard’ ‘higher’ or ‘freedom food’ and batches which were slaughtered in 90 days intervals between 1st September 2011 and 31st August 2013.

Univariate analysis was first carried out, followed by multivariate analysis to explore the combined effect of multiple factors on the odds of colonization at high levels (>123000cfu/g). Four multivariate models were built.

1. ‘biosecurity model’ a random effects logistic model was used to compare the odds of colonization between batches from farms with enhanced biosecurity (model batches) and batches from farms with standard biosecurity (control batches 1). The model controlled for the potential effect of harvest occasion (thinning vs. depopulation) 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).
2. ‘risk factors within high biosecurity farms model’ a random effects logistic model was used to compare the odds of colonization between batches at different harvest occasion while controlling for the potential effect of type of hybrid, empty days between flocks and season. As for model 1, model 2 also accounted for within-farm clustering. Only batches from model farms were used in this model as data on husbandry factors were only available for model farms.
3. ‘thinning practice model’ a random effects logistic model was used to compare the odds of colonization at depopulation between batches where partial depopulation was conducted and

batches without partial depopulation. This model controlled for potential effect of season and within-farm clustering and was limited to model batches only.

4. 'A Company's five farms model' Conditional logistic regression was used to compare the odds of colonization between batches from five farms (A-E) with enhanced biosecurity and batches from five farms with standard biosecurity (control batches 3). The model controlled for harvest occasion and season, and accounted for within-farm clustering.

Control batches 2 were not included in the multivariate models due to the data for only 16 batches at thinning and 14 at depopulation.

Estimation of Population Attributable Fractions (PAFs)

We utilized the estimates of the strength of the association between i) enhanced biosecurity, ii) partial depopulation and iii) hybrid type with odds of colonization at high levels (obtained from the models mentioned above), to estimate the proportion of heavily colonized batches that could be attributed to each of these factors (PAFs). 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. Assumptions were made as to the proportion of the total broiler population currently "exposed" to each of the 3 individual factors (i.e. all flocks are under standard biosecurity, 30 % of the flocks are of hybrids with low colonization results and 90% of batches are thinned; these are believed to be reasonable values for the UK broiler population).

The ORs obtained from the regression models were converted to adjusted relative risk (RRa) values [22] and used to estimate population attributable fraction (PAF) [23, 24].

$$RRa = OR / [(1-\text{Risk at baseline}) + (\text{Risk at baseline} * OR)] \quad \text{(eq. 1)}$$

PAF values were estimated as

$$PAF = Pd*(RRa-1)/RRa \quad \text{(eq. 2)}$$

and where Pd is the percentage of batches exposed to factors among highly colonized batches.

RESULTS

The identified 95% C.I. 2.09 – 3.68 of differences between caeca and neck skin results on log_{10} scale suggests that the batches positive in neck skin >1000 cfu/g (3 log_{10}) were colonized in caeca with results of at least 5.09 log_{10}.

Overall, 58.6% of all the studied batches were heavily colonized (>123000 cfu/g in pooled caecal samples) (Table 1). The proportion of colonized batches exhibited a seasonal pattern, with peaks during the summer period (Figure 1, Figure 2).

Univariate analysis

In the univariate analysis, all the factors under study, except the poultry company of origin, were significantly (P<0.05) associated with colonization at high levels (Table 2).

Multivariate analysis

Biosecurity model

Enhancement of biosecurity modified the effect of harvesting at thinning vs. at depopulation and vice versa (Table 3). Enhancement of biosecurity reduced the odds of colonization when harvesting took place at thinning (25% of the odds of infection of a standard biosecurity batch harvested at thinning) but the effect was markedly reduced when harvesting took place at the end of the cycle (47% of the odds of a standard biosecurity batch harvested at depopulation). A high proportion (72.9%) of batches raised under standard biosecurity was already colonized at the time of thinning. Only 41.7% of batches raised under enhanced biosecurity were colonized at thinning. This proportion increased to 64.7% when harvesting took place at depopulation.

The model results confirm the role of season. The likelihood of batch colonization was higher in the summer.

Risk factors within high biosecurity farms model

In farms with enhanced biosecurity, batches at depopulation had three times higher odds of colonization than batches at thinning (Table 4). Compared to the baseline hybrid (Ross 308), batches of Cobb 500 had 53% of the odds of high colonization. The mixed Cobb 500 & Ross 308 had three times higher odds of colonization compared to Ross 308. The sheds that were kept empty for up to 1 week were less likely to produce highly colonized batches; OR 0.69 (95% C.I. 0.49 – 0.96) than batches grown after a 1-2 week empty period. An empty period between flocks in of more than 3

weeks was associated with 3 times higher odds of colonization than the baseline group of 1-2 weeks empty period. Batches which had experienced a short period (1-3 days) between thinning and depopulation had half the odds of colonization >123000 cfu/g compared with batches experiencing a period of 7-9 days. There is no statistical evidence to differentiate the results of Ross 308 from JA 87, Ross 708 or the mix of Cobb 500 & Ross 308.

Thinning practice model

In farms with enhanced biosecurity, flocks that were thinned had more than twice (2.63) the odds of colonization at depopulation than flocks that were not thinned (Table 5).

A company's five farms model

The results of comparing the odds of colonization in batches from five model farms matched to batches from the third group of control farms are presented in Table 6. The results confirmed the protective effect of enhanced biosecurity on batch colonization, the increased odds of colonization at depopulation and the seasonality of batch colonization.

Sensitivity analysis

In order to assess the impact of the chosen cut-off, we repeated all univariate and multivariate analyses using a lower threshold (1000 cfu/g) for classification of high-colonization based on caeca results. The result of this different cut-off was that 11.4% of batches were re-classified as highly-colonized. However we obtained very similar results for the risk factor analysis.

Population attributable fractions (PAF)

Under the assumptions that identified risk factors have a causal association with the colonization of poultry batches and that the above estimates provide an unbiased measure of the association between the studied exposures and colonization, the following estimates were made:

If all batches in the UK were raised under enhanced biosecurity an estimated 32.0% (95% C.I. 16.0%-41.0%) of colonized batches in the population would be avoided (Figure 3). This is under the assumption that no UK farms operate under enhanced biosecurity (with the exception of model farms in this study) in 2013.

If none of the batches were subject to thinning then an estimated 33.0% (95% C.I. 14.0%-44.0%) of highly colonized batches could be avoided (Figure 4). This value assumes that thinning is currently practised in 90% of batches (as observed in this study).

If all batches were of the hybrid types associated with a lower risk, between 4.0% and 27.0 % of batch colonization could be prevented (Figure 5). In this study, more than 70.0% of batches were from those hybrids associated with higher risk of colonization.

Interventions against different factors could be introduced simultaneously. We estimate that approximately 30% (95% C.I. 13.0% - 37.0%) of highly colonized batches could be avoided in a hypothetical scenario of successfully enhancing biosecurity in half of the batches, avoiding thinning in a third of batches in which it is currently practiced and shifting to hybrids with a lower risk of colonization in at least 30.0% of the batches being at high risk.

DISCUSSION

This study analyzed the impact of enhanced biosecurity measures and selected husbandry factors on campylobacter colonization of broiler batches. We proposed a threshold for high colonization in caeca (>123000cfu/g) by correlating caecal and neck skin results and considering the established cut-off for high-risk group in neck skin.

Effect of Biosecurity

The results of the analyses undertaken provide strong evidence that enhanced biosecurity has a protective effect on batch colonization at thinning, reducing the odds of high colonization by between 53.0% and 86.0%. At the time of depopulation, the effect of increased biosecurity is considerably lower. The strong association between enhanced biosecurity and colonization at the time of thinning and the subsequent attenuation of this effect at the time of total depopulation could indicate that enhanced biosecurity is more effective at delaying than preventing colonization.

Thinning practice

It is likely that thinning itself can be considered to directly counter the protective effects of enhanced biosecurity. That practice 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 [15, 18] and is also identified in this study: flocks that had been partially depopulated (thinned) experienced a two times higher odds of colonization at depopulation than batches in which partial depopulation had not been practised. The fact that thinning was applied to 90% of batches included in this study and the strong financial motivation of the practice suggest that ceasing it

completely may not be feasible in the UK, since it would require additional investments in new poultry houses.

Our findings supporting a protective effect of farm hygiene measures on batch colonization are in agreement with previous studies in the Netherlands [25], the UK [26] and Denmark [27]. Other studies in countries such as Norway and Iceland [28] indicated an unpredictable effect of hygienic measures on farm and reported conflicting evidence.

Other risk factors

There was evidence of an association between the number of empty days between flocks and colonization: batches for which the shed had been kept empty less than a week appear to be at lower risk (83.0%) of colonization. The batches processed after a prolonged empty period of more than 21 days had a 42.0% increase in risk when compared with a period of 8 – 14 days. Previous studies have also identified an association between the length of the empty period between flocks [29] and potential for re-infection from the contaminated environment [30]. A prolonged empty period between flocks increases the probability of the shed becoming contaminated from the environment by the time when new birds are introduced.

A short period (1-3 days) between thinning and depopulation was also associated with a lower risk of colonization compared to batches for which the period between thinning and depopulation was 7-9 days. The results support the existence of differences in campylobacter colonization between the hybrids; these may be due to a biological characteristic of the birds, differences in the length of the cycle, growth rates, age of harvest or unmeasured factors associated with the type of hybrid such as diet or specific husbandry practices. Previous experimental studies showed a little impact of broiler breed to the susceptibility of chicken to *C. jejuni* colonization, but it has been reported that in fast-growing breeds the inflammatory response remains elevated for longer [31].

As expected, the risk of colonization exhibits a strong seasonality, with batches raised during winter at significantly lower risk of colonization. The effect of season on colonization of batches has been extensively reported and tentatively attributed to the ability of *Campylobacter* spp. to decay or transform in cold conditions into a viable but nonculturable (VBNC) state which has the potential for lengthy survival. Other potential seasonal effects include flies as potential carriers [32, 33] and seasonal changes in farm practices [34, 35].

Differences that are not explained by the studied factors in the actual counts of campylobacter might be attributed to additional factors such as the dose of exposure, effectiveness of the transmission, the time elapsed from infection to slaughter and individual susceptibility including the influence of stress factors.

The estimated PAFs suggest that one third 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 a 4.0% and 27.0% reduction in the proportion of highly colonized batches. The expected effects of interventions (PAFs) are based on estimates obtained from the study batches and assume causal association between exposure and colonization. Extrapolations should be made with caution, however, they provide an indication of the extent to which interventions at farm level can mitigate campylobacter colonization in broiler chickens and as a result human exposure to *Campylobacter* spp. Preventing high colonization in one third of chicken batches by improving biosecurity has the potential to avert 7-10% of human cases attributed to consumption of chicken meat and drop the number of cases attributed to chicken reservoir as whole by approximately one quarter, assuming that the EFSA source attribution model [9] was correct. 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 excluded. 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. We have not evaluated the performance of different laboratories in the study. However, we believe that the use of standardized and well-known methodology reduces potential variation between the laboratories. The batches positive in caeca do not necessarily correlate perfectly with batches positive in neck skin. However, high colonization in caeca is expected to result in high positive results in neck skin. The PAF values are based on estimates of strength of association and of frequency of exposure obtained from poultry

batches grown in a non-probabilistic sample of farms and under the assumption of causal relationship between exposure and colonization. The values could be interpreted as an a-priori expectation of the likely effect of potential interventions. The formal assessment of effectiveness of different interventions would require a randomized control trial. Despite these limitations, it seems unlikely that the main findings of the study are due to these potential biases.

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 at retail. It also shows a potential to mitigate the risk of heavily contaminated chicken reaching the consumer by enhancing biosecurity in combination with other measures further along the poultry chain maximizing the effectiveness of intervention. The existence of 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.

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 foodstuffs 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. [36]. Studies in the Netherlands [37] and Nordic countries [38] propose the implementation of threshold levels for batch colonization at the end of slaughter. The results of this study justify the implementation of an intervention study to confirm and quantify the impact of combined changes to biosecurity and thinning including monitoring beyond the abattoir.

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Declaration of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides.

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Table 1 Number and proportion of batches found to be colonized at different levels in pooled caecal samples (results from 2314 batches included in the UK poultry industry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013).

Results	**at thinning**				**at depopulation**				
	Control farms 1 (%)	Control farms 2 (%)	Control farms 3 (%)	Model Farms (%)	Control farms 1 (%)	Control farms 2 (%)	Control farms 3 (%)	Model Farms (%)	**TOTAL** (%)
1 to <100cfu/g	21	4	29	338	23	0	11	191	**617**
	(10.6)	(25.0)	(43.3)	(41.2)	(11.5)		(15.9)	(20.6)	**(26.7)**
100 to 1000 cfu/g	1	0	1	32	0	1	1	41	**77**
	(0.5)		(1.5)	(3.9)		(7.1)	(1.4)	(4.4)	**(3.3)**
>1000 cfu/g	177	12	37	450	177	13	57	697	**1,620**
	(88.9)	(75.0)	(55.2)	(54.9)	(88.5)	(92.9)	(82.6)	(75.0)	**(70.0)**
>1000 to ≤123000	32	2	2	108	17	3	3	96	263
cfu/g	(16.1)	(12.5)	(3.0)	(13.2)	(8.5)	(21.4)	(4.3)	(10.3)	(11.4)
>123000	145	10	35	342	160	10	54	601	1357
cfu/g	(72.9)	(62.5)	(52.2)	(41.7)	(80.0)	(71.4)	(78.3)	(64.7)	(58.6)
TOTAL	**199**	**16**	**67**	**820**	**200**	**14**	**69**	**929**	**2314**

Table 2 Univariate associations between potential risk factors and *Campylobacter* spp. colonization at high level (>123000 cfu/g in pooled caecal samples; results from 2314 batches included in the UK poultry industry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013).

Variable	Categories	Number (%) of Batches >123000cfu/g	Number of Batches ≤123000cfu/g	p-value (chi^2)[i]
Harvest occasion	Thinning	532 (48.3)	570	<0.001
	Depopulation	824 (68.0)	388	
Biosecurity	Model Farms	943 (53.9)	806	<0.001
	Control farms 1	304 (76.2)	95	
	Control farms 2	20 (66.7)	10	
	Control farms 3	89 (65.4)	47	
Welfare in model farms	Standard	588 (53.5)	512	0.038
	Higher	305 (53.0)	271	
	Freedom Food[ii]	50 (68.5)	23	
Hybrid in model farms	Cobb 500	183 (48.4)	195	0.001
	Cobb 500& Ross 308	18 (72.0)	7	
	Ross 308	613 (54.5)	511	
	Ross 708	69 (50.4)	68	
	JA 87	57 (70.4)	24	
Empty days in model farms	1-7 days	233 (51.2)	218	<0.001
	8-14 days	585 (54.4)	491	
	15-21 days	57 (48.3)	61	
	22-47 days	35 (72.9)	13	
	na[iii]	446 (71.8)	175	
Days from thinning to depopulation in model farms	1-3 days	143 (48.8)	150	<0.001
	4-6 days	344 (54.4)	288	
	7-9 days	215 (58.0)	156	
	10-12 days	99 (57.2)	74	

	13-18 days	60 (60.6)	39	
	na	495 (66.4)	251	
Processors dealing with batches of control farms 1	Q	54 (77.14)	16	0.088
	R	58 (79.5)	15	
	S	99 (81.8)	22	
	T	93 (68.9)	42	
Practice of partial depopulation in model farms	Thinning had been practised	555 (66.6)	279	<0.001
	Thinning had not been practised	46 (48.4)	49	

[i] chi^2 test on (r x c) tables;

[ii] in addition to the specific welfare conditions the category requires rearing of hybrid JA 87

[iii] the information is not available

Table 3 Results of a random effects logistic regression (Regression Model 1 'biosecurity model') of enhanced biosecurity, harvest occasion and sampling period on batch colonization (defined as >123000 cfu/g in pooled caecal samples). Results from a total of 1687 batches sampled between 16th April 2012 and 31st August 2013 included in the UK poultry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013.

Factors	OR (95% C.I.)	P-value
Biosecurity		
Standard (control farms 1)	1.00	
Enhanced (model farms)	0.25 (0.14-0.47)	<0.001
Harvest occasion		
Thinning (T)	1.00	
Depopulation (D)	1.68 (0.93-3.03)	0.086
Interaction between biosecurity & harvest occasion		
Model farm & Depopulation	1.85 (0.98-3.50)	0.059
Effect of Depopulation:		
- in model farm	3.10 (2.43-3.96)	
- in control farms1	1.68 (0.93-3.03)	
Effect of enhanced biosecurity		
- at thinning	0.25 (0.14-0.47)	
- at depopulation	0.47 (0.25-0.89)	
Sampling period		
16 Apr – 31 May 2012	3.56 (2.26-5.61)	<0.001
1 June – 31 Aug 2012	5.91 (4.00-8.73)	<0.001
1 Sept - 30 Nov 2012	1.21 (0.86-1.72)	0.278
1 Dec - 28 Feb 2013	1.00	
1 Mar - 31 May 2013	1.09 (0.77-1.54)	0.619
1 June - 31 Aug 2013	3.04 (2.11-4.38)	<0.001
Constant	1.60 (0.88-2.88)	0.121
standard deviation of random effects	0.40 (0.25-0.63)	
Interclass correlation coefficient (rho)	0.05 (0.02-0.11)	

Table 4 Results of random effects logistic regression (Regression Model 2 'risk factors within high biosecurity farms model') investigating the contribution of selected factors in model farms to *Campylobacter* spp. colonization (defined as >123000 cfu/g in pooled caecal samples). Results from a total of 1510 batches sampled between 16th October 2011 and 31st August 2013 in 16 farms with enhanced biosecurity included in the UK poultry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013.

Factors	OR (95% C.I.)	P-value
Harvest occasion		
Thinning	1.00	<0.001
Depopulation	3.30 (2.61-4.18)	
Type of hybrid		
Cobb 500	0.53 (0.31-0.89)	0.017
Cobb 500 & Ross 308	3.23 (1.08-9.63)	0.035
JA 87	1.27 (0.42-3.85)	0.670
Ross 308	1	
Ross 708	0.68 (0.35-1.33)	0.266
Empty days		
up to 1 week	0.69 (0.49-0.96)	0.026
1 - 2 weeks	1	
2 – 3 weeks	0.90 (0.57-1.42)	0.645
> 3 weeks	3.03 (1.14-8.07)	0.027
Days to depopulation		
1 – 3 days	0.57 (0.36-0.90)	0.016
4 – 6 days	0.85 (0.60-1.18)	0.337
7 - 9 days	1	
10 – 12 days	0.85 (0.53-1.38)	0.521
13 – 18 days	0.48 (0.24-0.99)	0.047
Sampling period		
16 Oct – 30 Nov 2011	0.74 (0.36-1.51)	0.414
1 Dec - 29 Feb 2012	0.86 (0.54-1.37)	0.526
1 Mar – 31 May 2012	1.99 (1.29-3.08)	0.002
1 June - 30 Aug 2012	7.74 (4.76-12.59)	<0.001
1 Sept - 30 Nov2012	0.92 (0.59-1.42)	0.694
1 Dec - 28 Feb 2013	1	
1 Mar – 31 May 2013	1.13 (0.73-1.76)	0.581
1 June - 30 Aug 2013	4.18 (2.62-6.69)	<0.001
Constant	0.61 (0.37-1.01)	0.053
standard deviation of random effects	0.51 (0.29-0.90)	
Interclass correlation coefficient (rho)	0.07 (0.03-0.20)	

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Table 5 Results of random effects logistic regression (Regression Model 3 'thinning practice model') investigating the effect of partial depopulation (thinning) on *Campylobacter* spp. colonization (defined as >123000 cfu/g in pooled caecal samples) at depopulation. Results from a total of 888 batches sampled between 16th October 2011 and 31 August 2013 included in the UK poultry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013.

Factors	OR (95% C.I.)	P-value
Practice of thinning		
The flock had not been partially depopulated (78 batches)	1.00	0.004
The flock had been partially depopulated (thinned) (810 batches)	2.43 (1.34-4.42)	
Sampling period		
16 Oct – 30 Nov 2011	0.63 (0.20-1.37)	0.245
1 Dec - 29 Feb 2012	0.53 (0.30-0.93)	0.028
1 Mar – 31 May 2012	2.52 (1.40-4.43)	0.001
1 June - 30 Aug 2012	4.90 (2.60-9.21)	<0.001
1 Sept - 30 Nov2012	0.88 (0.50-1.49)	0.624
1 Dec - 28 Feb 2013	1.00	
1 Mar – 31 May 2013	1.69 (0.90-2.93)	0.064
1 June - 30 Aug 2013	1.57 (0.90-2.71)	0.101
Constant	0.66 (0.30-1.36)	0.263
standard deviation of random effects	0.47 (0.26-0.85)	
Interclass correlation coefficient (rho)	0.06 (0.02-0.18)	

Table 6 Results of a conditional logistic regression (Regression Model 4, A company's five farms model) of enhanced biosecurity and other factors on batch colonization (defined as >123000 cfu/g in pooled caecal samples). Results from a total of 712 batches sampled between 16th October 2011 and 31st August 2013 included in the UK poultry study on enhanced biosecurity and campylobacter colonization; UK, 2011-2013.

Factors	OR (95% C.I.)	P-value
Biosecurity		
Standard (control farms 1)	1	<0.001
Enhanced (model farms)	0.32 (0.20-0.52)	
Harvest occasion		
Thinning (T)	1	<0.001
Depopulation (D)	2.87 (2.00-4.12)	
Sampling period		
16 Oct – 30 Nov 2011	0.71 (0.30-1.68)	0.437
1 Dec - 29 Feb 2012	0.62 (0.31-1.25)	0.178
1 Mar – 31 May 2012	6.99 (3.63-13.46)	<0.001
1 June - 30 Aug 2012	19.90 (9.21-43.00)	<0.001
1 Sept - 30 Nov2012	1.08 (0.57-2.05)	0.813
1 Dec - 28 Feb 2013	1	
1 Mar – 31 May 2013	2.22 (1.19-4.13)	0.012
1 June - 30 Aug 2013	5.90 (3.05-11.42)	<0.001

Fig. 1. Seasonal variation in Campylobacter colonization of batches in model farms. Colonized batches are those with >123 000 c.f.u./g in pooled faecal samples obtained either at thinning (T) or at depopulation (D).

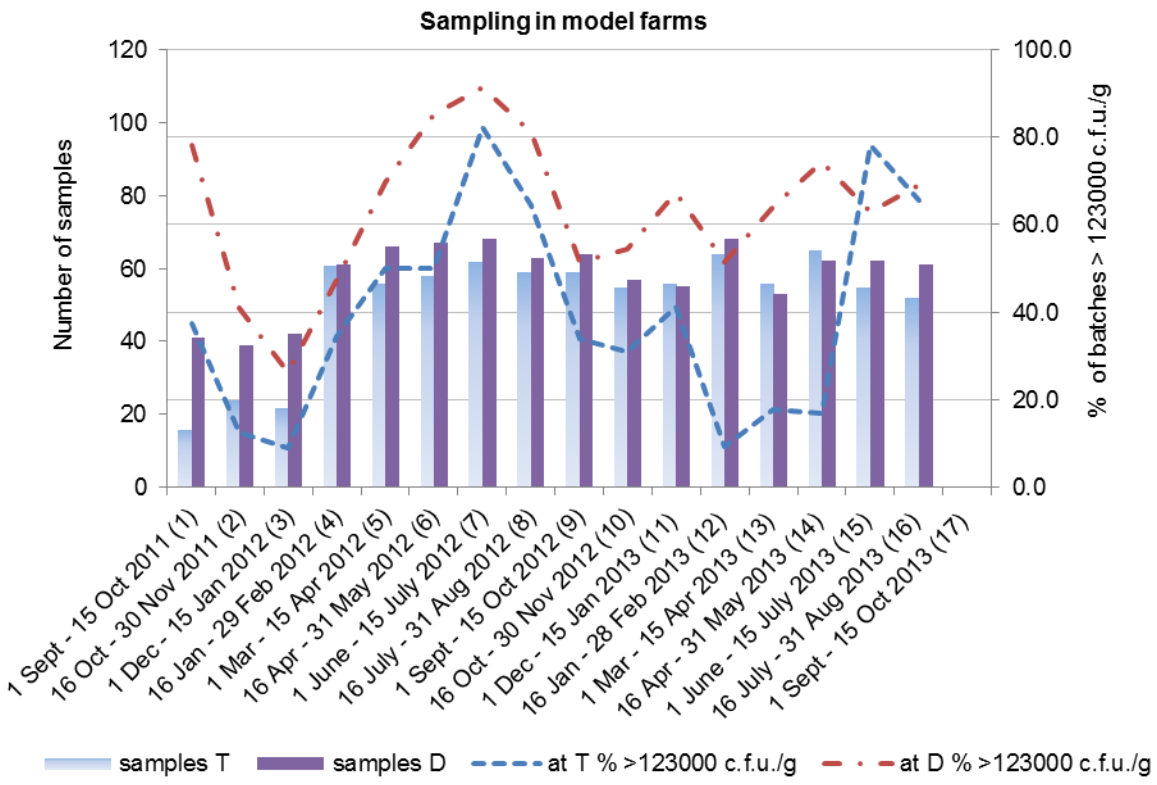

Fig. 2. Seasonal variation in Campylobacter colonization of batches in control farms. Colonized batches are those with >123 000 c.f.u./g in pooled faecal samples obtained either at thinning (T) or at depopulation (D).

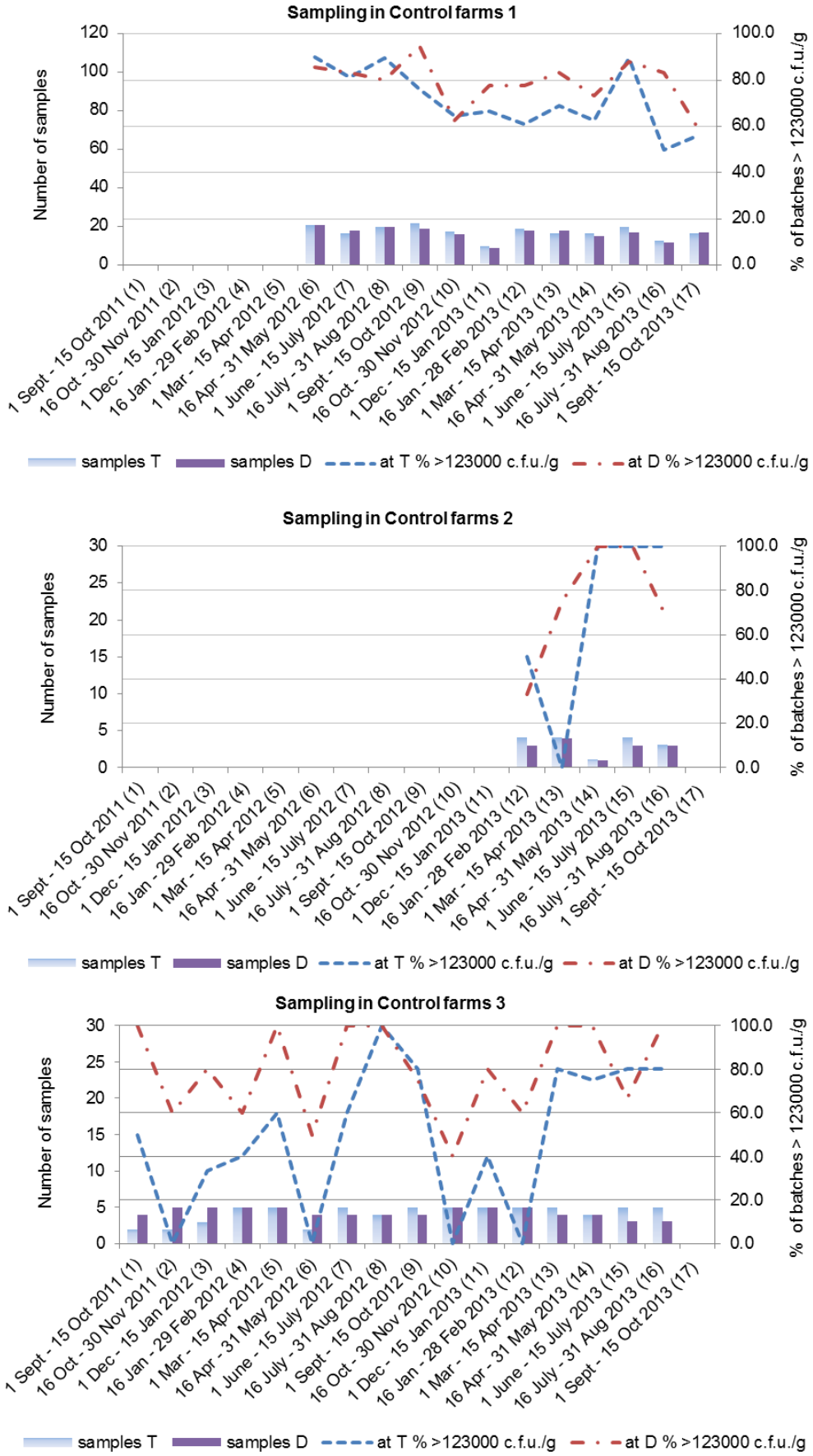

Fig. 3. Population attributable fraction (PAF) of the effect of enhanced biosecurity on batch colonization at thinning and depopulation.

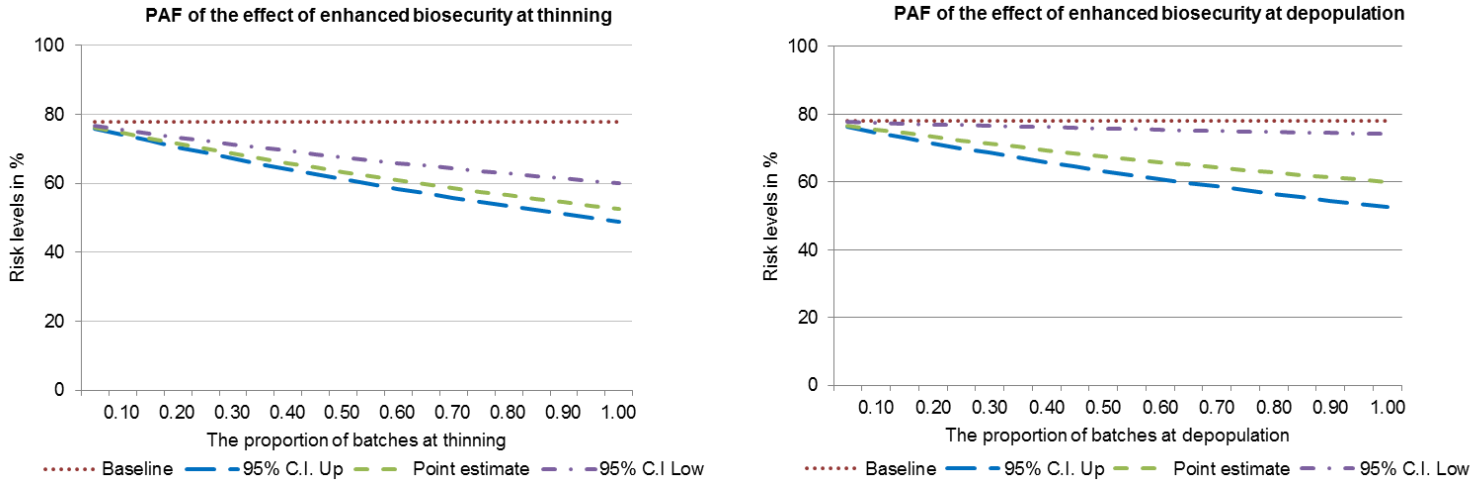

Fig. 4. Population attributable fraction (PAF) of the effect of the practice of thinning on batch colonization in model farms.

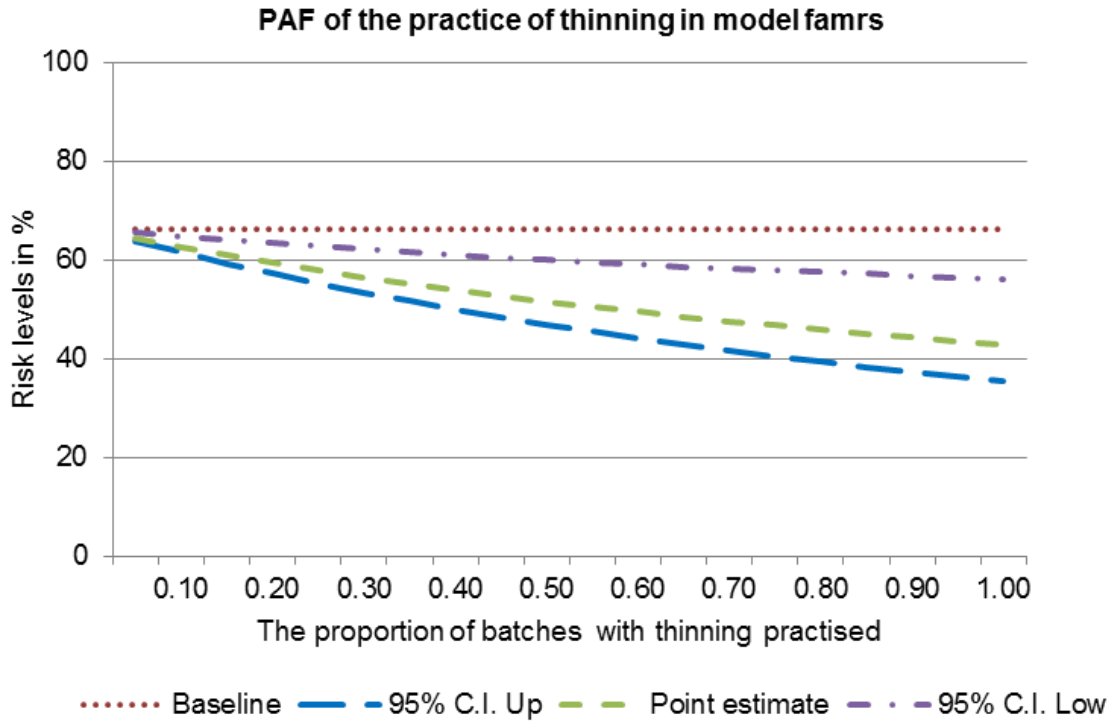
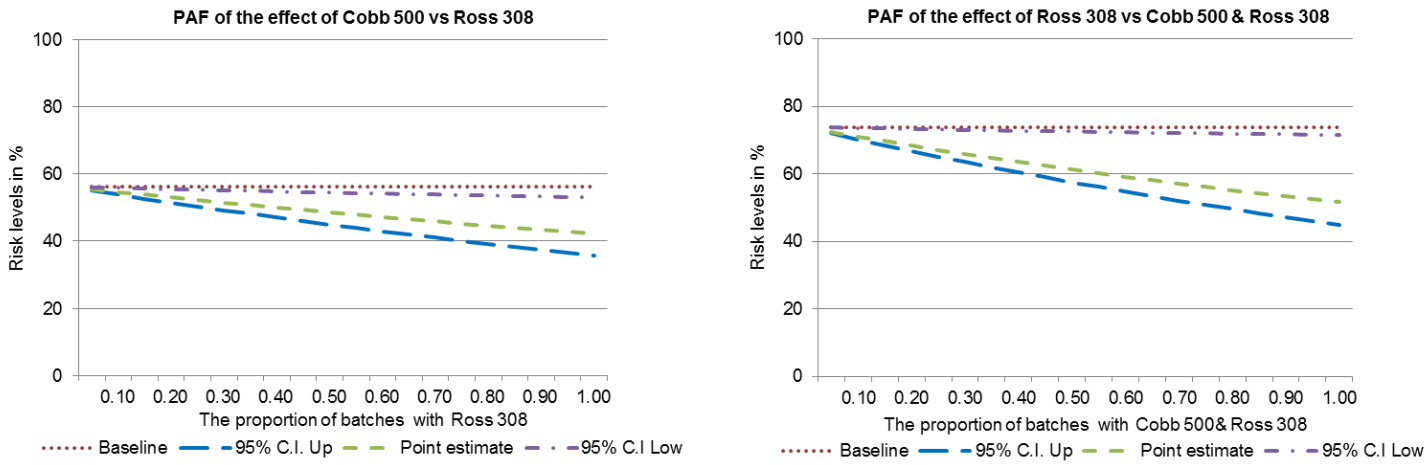
Fig. 5. Population attributable fraction (PAF) of the effect of hybrids on batch colonization in model farms.