

# Data collected for risk assessment and economics Deliverable 4.2.1

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# Introduction

This report *Data collected for risk assessment and economics* is Deliverable 4.2.1 of the CamCon project funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement no. 244547. The deliverable was due and delivered Month 58, April 2015. Data and information presented in the report were provided by several CamCon project participants; the National Veterinary Institute in Norway, LEI Wageningen University and Research Center, the Netherlands, University of Liverpool in the UK, the National Veterinary Research Institute in Poland, the the National Food Institute, the Technical University of Denmark.

The report includes data collected for work package 4 (WP4); risk assessment and economics.

The objective of this WP was to identify cost-effective interventions to control *Campylobacter* at farms in different countries across Europe and to compare these with cost-effective interventions at and after slaughter identified in other studies. Together with the findings of CamCon WP1 in which significant risk factors are identified, this enables us to investigate the effect on human incidence of campylobacteriosis and the cost-effectiveness and cost-utility of different intervention strategies under different climate zones and production circumstances.

The report includes the following information:

- Results of a literature survey on *Campylobacter* in the broiler chain (2007-2013).
- Results of analyzing the correlation between *Campylobacter* in caeca and meat on data provided by partners in Norway, the UK, Spain and Poland.
- Results of analyzing the impact of interventions (thinning, slaughter age, fly screens) that could not be extracted from the risk factor study in CamCon WP1.
- *Campylobacter* prevalences in broiler flocks in Denmark, the Netherlands, Norway, Poland, Spain and the UK.
- Costs of interventions.

### Acknowledgements

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 244547.

The authors wish to thank all project participants for their contributions to the report. Also the efforts made by participating broiler farmers, staff at involved broiler companies, and technical staff of the participating institutions are gratefully acknowledged.

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# 1. Literature survey on *Campylobacter* in the broiler chain

Mona Torp, Norwegian Veterinary Institute, Norway

With the aim to identify data to support risk assessment activities in WP4, a literature survey was performed early 2014. The information searched for included new quantitative *Campylobacter* data in the broiler chain. For *Campylobacter* prevalence and concentration data as well as production data (topics 1-6) preferably European data (and especially UK, NL, DK, NO, PL, ES) were prioritized. Regarding topics 7-8 data from any country were looked for. Relevant literature including research papers and national reports were searched from 2007 and onwards using PubMed, Medline and Google.

Table 1 shows the list of data that were searched for in the literature survey. References that were already known by the risk assessors were listed beforehand (Appendix 1) to avoid searching for known information. The results of the survey are summarized in table 2.

# Table 1. Data on Campylobacter searched for in the literature survey (=topics in table 2)

1. Data on flock prevalences (preferably per month/season)

- at slaughter, the % of flocks that is found to be infected when entering the slaughter process
- a week (between 3-10 days) prior to slaughter. Preferably in combination with data on scheduled slaughter (thinning)
- 2. Within flock prevalence at slaughter (preferably per month/season)
- Point estimate of the % infected birds within a flock, or a distribution of within flock prevalences between flocks
- 3. Data on caecal and/or faecal concentrations in broilers (preferably per month/season)
- Distribution of the means per flock (with different means for different flocks, indicate the number of flocks, otherwise one mean)
- Within flock variation (e.g. the standard deviations in concentrations for different flocks)
- 4. Concentrations of Campylobacter on the exteriors immediately after killing/deheading.
- Distribution of the means per flock (if different means for different flocks, the number of flocks is indicated; otherwise one mean)
- Within flock variation (e.g. the standard deviations in concentrations for different flocks)
- 5. Concentrations of Campylobacter on skin samples or meat samples after chilling or at retail
- Distribution of the means per batch (if different means for different batches, the number of batches is indicated; otherwise one mean)
- Within batch variation (e.g. the standard deviations in concentrations for different batches)

6. Production data

- Ratio of chickens (not flocks) sent to frozen-slaughtering versus chill-slaughtering
- Ratio of chill-slaughtered broilers that are sent to parting versus whole broiler product
- Ratio of frost-slaughtered broilers that are sent to parting versus whole broiler product
- Ratio of the chilled parts with skin versus without skin
- Ratio of the frozen parts with skin versus without skin

7. Studies associating concentrations in caeca / faeces with concentrations on the meat of the same flocks

- One such study is the basis for the EFSA model (EFSA 2011; Reich et al 2008), it is good to be sure which are available now. See also Nauta et al. 2009.
- 8. Campylobacter in broiler meat risk assessments.

Table 2. Result of the literature survey on new information on Campylobacter in the broiler chain (2007-2013).

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	Table <sup>b</sup>	Fig. <sup>c</sup>	Comments
Boysen L, Nauta M, Ribeiro Duarte AS, Rosenquist H.	Human risk from thermotolerant Campylobacter on broiler meat in Denmark.	Int J Food Microbiol. 2013;162(2):1 29-34.	2013	DK	8	Semi-quantitative Campy broiler meat. Risk reduction estimation	Domestic and imported frozen and chilled meat at retail. Prev. and conc.	10	Yes	Yes	Some product data from 2001 - 2010
Christensen BB, Nauta M, Korsgaard H, Vedel Sørensen AI, Rosenquist H, Boysen L, Perge A, Nørrung B.	Case-by-case risk assessment of broiler meat batches: An effective control strategy for Campylobacter.	Food Control. 2013;31:485- 90.	2013	DK	8	Basis for the RA-model by batch	> 3000 batches, domestic and imports, frozen and chilled	4	Yes	Yes	Some production data from 2005
Kudirkiene E, Buneviciene J, Serniene L, Ramonaite S, Olsen JE, Malakauskas M.	Importance of the producer on retail broiler meat product contamination with Campylobacter spp.	Journal of the science of food and agriculture. 2013;93(9):22 93-8.	2013	LT	5	Campy prev. and concentrations. Fla-RFLP typing. Meat bought at retail.	312 broiler meat products (wings and drumsticks) from 3 producers. Results per month.	1	Yes	Yes	Results given per producer. No flock information or production method.
Rosenquist HB, L.; Krogh, A.L.; Nygaard-Jensen, A.; Nauta, M.	Campylobacter contamination and the relative risk of illness from organic broiler meat in comparison with conventional broiler meat.	Int J Food Microbiol. 2013;162:226 – 30	2013	DK	8 (5)	Campy on carcasses after chilling. Risk estimation	Organic and conventional broiler meat. Carcass prevalence and conc. Four seasons	1	Yes	Yes	Concentration data pr batch not presented
Signorini ML, Zbrun MV, Romero-Scharpen A, Olivero C, Bongiovanni F, Soto LP, et al.	Quantitative risk assessment of human campylobacteriosis by consumption of salad cross-contaminated with thermophilic Campylobacter spp. from broiler meat in Argentina.	Preventive veterinary medicine. 2013;109(1- 2):37-46.	2013	Arg enti na	8	Campy; MPN. Carcass rinse, slaughter and retail. Model development risk assessment for cross contamination	Consumer handling, consumption of poultry meat with salad. 30 carcasses from slaughter, 30 samples from retail	1	Yes	Yes	Table with model input parameters

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	Table <sup>b</sup>	Fig. <sup>c</sup>	Comments
Chowdhury S, Sandberg M, Themudo GE, Ersboll AK.	Risk factors for Campylobacter infection in Danish broiler chickens.	Poultry science. 2012;91(10): 2701-9.	2012	DK	1	Campy prevalence fecal samples. Risk factors investigated	2835 flocks, 187 farms. Fecal samples from socks 7 - 10 days before slaughter/thinningSeasona I variation	1	Yes	No	Table showing seasonal variation and other variables.
Habib I, Berkvens D, De Zutter L, Dierick K, Van Huffel X, Speybroeck N, et al.	Campylobacter contamination in broiler carcasses and correlation with slaughterhouses operational hygiene inspection.	Food microbiology. 2012;29(1): 105-12.	2012	BE	5	Campy post-chill carcasses from 9 slaughterhouses. Prev. and conc. Risk factors at slaughterhouse.	Neck skin and breast skin in 389 carcasses. One carcass per batch. EU baseline 2008. Risk factors from official inspection at slaughterhouse	1	Yes	Yes	Table with distribution of contamination per slaughterhouse. No information about flocks.
Lawes JR, Vidal A, Clifton-Hadley FA, Sayers R, Rodgers J, Snow L, et al.	Investigation of prevalence and risk factors for Campylobacter in broiler flocks at slaughter: results from a UK survey.	Epidemiology and infection. 2012;140(10): 1725-37.	2012	UK	1	Campy prev. in pooled caecal samples at slaughter. Risk factors at farm, season included.	1174 slaughter batches sampled in 2007- 2009. 33 abattoirs participated. Conventional, organic and free-range birds.	3	Yes	Yes	
Nauta MJ, Sanaa M, Havelaar AH.	Risk based microbiological criteria for Campylobacter in broiler meat in the European Union.	Int J Food Microbiol. 2012;158(3): 209-17.	2012	DK, FR, NL	8	Estimation of risk reduction of setting microbiol. criteria for Campy in broiler meat.	Risk reduction in 25 countries. Applying quantitative data from EU baseline 2008.	1	Yes	Yes	
Powell LF, Lawes JR, Clifton-Hadley FA, Rodgers J, Harris K, Evans SJ, et al.	The prevalence of Campylobacter spp. in broiler flocks and on broiler carcasses, and the risks associated with highly contaminated carcasses.	Epidemiology and infection. 2012;140(12): 2233-46.	2012	UK	1, 5	Campy prev. in pooled caecal samples at slaughter. Prev. and conc. on 1 broiler carcass post-chill per batch. Risk factors.	Neck skin from 400 carcasses/batches and ten pooled caecal samples from the same batches. EU baseline 2008. Risk association.	1	Yes	Yes	
Boysen L, Vigre H, Rosenquist H.	Seasonal influence on the prevalence of thermotolerant Campylobacter in retail broiler meat in Denmark.	Food microbiology. 2011;28(5): 1028-32.	2011	DK	?	Campy prev. in meat compared to season, origin of meat, flock prev. in the same periods. Estimations.	Number of samples given in diagram	7	No	Yes	Prevalence estimates given in figures.

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	Table <sup>b</sup>	Fig. <sup>c</sup>	Comments
Hayama Y, Yamamoto T, Kasuga F, Tsutsui T.	Simulation model for Campylobacter cross- contamination during poultry processing at slaughterhouses.	Zoonoses and public health. 2011;58(6): 399-406.	2011	Jap an	8	Individual-based simulation model, cross contamination	Influence of flock prevalence and campy per carcass before processing, and during different stages of the slaughtering process		No	Yes	Estimated prevalenc- and concentrations- data in the simulation model.
Hue O, Allain V, Laisney MJ, Le Bouquin S, Lalande F, Petetin I, et al.	Campylobacter contamination of broiler caeca and carcasses at the slaughterhouse and correlation with Salmonella contamination.	Food microbiology. 2011;28(5): 862-8.	2011	FR	7	Campy conc. in pooled caecal samples at slaughter. Concentr. on 1 broiler carcass post-chill per batch. Correlations.	Skin from neck and breast from 425 carcasses/batches and ten pooled caecal samples from the same batches. EU baseline 2008. Correlations.	1	Yes	Yes	See also O. Hue et al. 2010
Jorgensen F, Ellis- Iversen J, Rushton S, Bull SA, Harris SA, Bryan SJ, et al.	Influence of season and geography on Campylobacter jejuni and C. coli subtypes in housed broiler flocks reared in Great Britain.	Applied and environmental microbiology. 2011;77(11): 3741-8.	2011	UK	1, 2	Campy prev. in flocks at slaughter. Within-batch prevalence in positive flocks. Risk factors at farm level. Typing results.	797 flocks from 211 farms. Up to 30 caeca per batch investigated separately. Prev. of C. jejuni and C. coli. Dec 2003 - March 2006.	2,5	Yes	Yes	Within-batch prev. and prev. by region and month shown in figures.
Malher X, Simon M, Charnay V, Deserts RD, Lehebel A, Belloc C.	Factors associated with carcass contamination by Campylobacter at slaughterhouse in cecal- carrier broilers.	Int J Food Microbiol. 2011;150(1):8- 13.	2011	FR	7	Caecal counts and neck-skin counts from campy-pos batches. Risk factors .	Samples from 108 batches in 3 slaughterhouses May to August 2009.	mont hs	Yes	Yes	Risk factors presented in tables, caecal and skin counts in figures.
Nauta M, Christensen B.	The impact of consumer phase models in microbial risk analysis.	Risk analysis : an official publication of the Society for Risk Analysis. 2011;31(2): 255-65.	2011	DK	8	Comparison of performance of published CPMs for Campy in broiler meat	8 published CPMs in an example of quantitative microbiol. risk assessment		Yes	Yes	
di Giannatale E, Prencipe V, Co- langeli P, Alessiani A, Barco L, Staffolani M,et al	Prevalence of thermotolerant Campylobacter in broiler flocks and broiler carcasses in Italy.	Veterinaria italiana. 2010;46(4): 405-23.	2010	IT	1, 5	Campy prev. in pooled caecal samples at slaughter. Prev. and concentr. on 1 broiler carcass post-chill per batch.	EU baseline 2008. 393 batches from 11 regions.	1	Yes	Yes	Full text in Italian

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	<b>Table<sup>b</sup></b>	Fig. <sup>c</sup>	Comments
Ellerbroek LI, Lienau JA, Klein G.	Campylobacter spp. in broiler flocks at farm level and the potential for cross-contamination during slaughter.	Zoonoses and public health. 2010;57(7- 8):e81-8.	2010	DE	1	Campy prev. in pooled faecal samples at farm. Prev. in transport crates, carcasses and final products. PFGE on isolates	Samples from 51 broiler flocks December to August 2001/2002. 1101 samples from 22 flocks during processing. Seasonal variation.	< 1	Yes	Yes	Table showing contam. results at different stations during processing. Other results in figures.
Hue O, Le Bouquin S, Laisney MJ, Allain V, Lalande F, Petetin I, et al.	Prevalence of and risk factors for Campylobacter spp. contamination of broiler chicken carcasses at the slaughterhouse.	Food microbiology. 2010;27(8): 992-9.	2010	FR	1, 5	Campy prev. in pooled caecal samples at slaughter. Prev. and concentr. on 1 broiler carcass post-chill per batch. Risk factors.	Skin from neck and breast from 425 carcasses/batches and ten pooled caecal samples from the same batches. EU baseline 2008. Risk association.	1	Yes	Yes	See also O. Hue et al. 2011
Jonsson ME, Norstrom M, Sandberg M, Ersboll AK, Hofshagen M.	Space-time patterns of Campylobacter spp. colonization in broiler flocks, 2002-2006.	Epidemiology and infection. 2010;138(9): 1336-45.	2010	NO	1	Campy prev. in pooled faecal/caecal samples. Samples at farm and at abattoir. Spatial relative risk maps. Geographical coordinates.	643 farms and 16523 flocks included n the study.	5	Yes	Yes	Clusters in table, other results in figures.
Nather G, Alter T, Martin A, Ellerbroek L.	Analysis of risk factors for Campylobacter species infection in broiler flocks.	Poultry science. 2009;88(6): 1299-305.	2009	DE	1	Campy prev. in pooled caecal samples at slaughter. Risk factors.	146 flocks at slaughter from May 2004 - April 2005. including conventional, organic and free-range farms	1	Yes	No	Seasonal variation in table.
Nauta M, Hill A, Rosenquist H, Brynestad S, Fetsch A, van der Logt P, et al.	A comparison of risk assessments on Campylobacter in broiler meat.	Int J Food Microbiol. 2009;129(2): 107-23.	2009	NL, UK, DK, DE, NO, NZ, CA	8	Comparison of risk assessment models					

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	Table <sup>♭</sup>	Fig. <sup>c</sup>	Comments
Albert I, Grenier E, Denis JB, Rousseau J.	Quantitative risk assessment from farm to fork and beyond: a global Bayesian approach concerning food-borne diseases.	Risk analysis : an official publi.cation of the Society for Risk Analysis. 2008;28(2): 557-71.	2008	FR	8	Bayesian techniques in two steps. Campy in broilers used as model organism			Yes	Yes	Tables with variates
Calistri P, Giovannini A.	Quantitative risk assessment of human campylobacteriosis related to the consumption of chicken meat in two Italian regions.	Int J Food Microbiol. 2008;128(2): 274-87.	2008	IT	8	Campy MPN from other investigation used. Data on qualitative and quantitative weekly consumtion of food products. Handling in domestic kitchen.	392 samples from carcasses and cut parts included. 2002/2003. Cross contamination in domestic kitchen assumed.	< 1	Yes	Yes	
Colles FM, Jones TA, McCarthy ND, Sheppard SK, Cody AJ, Dingle KE, et al.	Campylobacter infection of broiler chickens in a free-range environment.	Environmental microbiology. 2008;10(8): 2042-50.	2008	UK	1	Campy prev. at farm. Anal swabs before depopulation. 10 - 25 birds sampled per flock. MLST typing.	975 chickens from 64 free- range broiler flocks from 2 sites included. Samplingperiod February- december 2003.		Yes	Yes	
Nauta MJ, Havelaar AH	Risk-based standards for Campylobacter in the broiler meat chain.	Food Control. 2008;19:372 - 81.	2008	NL	8						How a farm to fork QMRA can be applied to guide the settings of risk- based standards for food safety
Hansson I, Forshell LP, Gustafsson P, Boqvist S, Lindblad J, Engvall EO, et al.	Summary of the Swedish Campylobacter program in broilers, 2001 through 2005.	Journal of food protection. 2007;70(9): 2008-14.	2007	SE	1, 2, 4, (5)	Campy prev. at farm., at slaughter, within flock prev. at slaughter. Concentrations neck skin, carcass.	A total of 70160 samples analyzed.	5	Yes	Yes	Several studies in one.
Johannessen GS, Johnsen G, Okland M, Cudjoe KS, Hofshagen M.	Enumeration of thermotolerant Campylobacter spp. from poultry carcasses at the end of the slaughter-line.	Letters in applied microbiology. 2007;44(1):92- 7.	2007	NO	2, 5	Campy within flock prev. at slaughter. Concentrations carcasses after chilling. AFLP fingerprinting.	51 carcasses from 17 flocks at one slaughterhouse during 5 days in August 2005		Yes	No	Campy status of flocks known on arrival slaughterhouse.

Authors	Titel	Ref	Year	Cou ntry	Top ic*	Methods	Some details	Years incl. <sup>a</sup>	Table <sup>b</sup>	Fig. <sup>c</sup>	Comments
Katsma WE, De Koeijer AA, Jacobs- Reitsma WF, Mangen MJ, Wagenaar JA.	Assessing interventions to reduce the risk of Campylobacter prevalence in broilers.	Risk analysis : an official publication of the Society for Risk Analysis. 2007;27(4): 863-76.	2007	NL	8	Development of model to describe transmission within broiler house and between broiler houses. Estimations.	Model to quantify campy prev. risk when the flock leaves the farm for processing.		Yes	Yes	As an input to a more comprehensive risk assessment.
Guerin MT, Martin W, Reiersen J, Berke O, McEwen SA, Bisaillon JR, et al.	A farm-level study of risk factors associated with the colonization of broiler flocks with Campylobacter spp. in Iceland, 2001-2004.	Acta veterinaria Scandinavica. 2007;49:18.	2007	Icel.	1	Prev. in pooled caecal samples at slaughter. Risk factors.	Prev. In samples from 1425 flocks. Seasonal variation. Subset of 792 flocks studied for risk factors.	4	No/Yes	No	Prev. only given for summer flocks
Prencipe V, Parisciani G, Calistri P, et al.	Thermotolerant Campylobacter in poultry meat marketed in the Abruzzo and Molise regions of Italy: prevalence and contamination level.	Veterinaria italiana. 2007;43(1): 167-74.	2007	IT	5	Prev. and MPN in poultry meat from small and large retailers.	From Dec June 2003, 392 samples from whole and sectioned chickens, loose and packaged products.	mont hs	Yes	Yes	
Barrios PR, Reiersen J, Lowman R et al.	Risk factors for Campylobacter spp. colonization in broiler flocks in Iceland.	Prev Vet Med. 2006; 74:264- 78	2006	lcel.	1	Prev. in pooled caecal samples at slaughter. Risk factors.	Prev. in samples from 1091 flocks. Seasonal variation.	3	No/yes	Yes	Seasonal flock prevalence given in figure.

\*, refers to table 1

a, number of years included

b, data available in tables

c, data available in figures

# 2. Campylobacter in caeca and on broiler carcasses in Norway

*Gro Johannessen, Norwegian Veterinary Institute, Norway (data) Maarten Nauta, Technical University of Denmark (data analysis)* 

With the aim to generate information on the relation between the concentration of *Campylobacter* in caeca and the concentration on meat, which is essential information for the risk assessment in WP4, samples of caeca and neck skin were collected from 10 *Campylobacter* positive flocks at two Norwegian slaughter plants (five flocks per plant) in the period May-October 2013 as part of the CamCon project.

From each flock caecal samples (n=25), neck skin samples (n=25) and carcass rinse samples were collected (n=5). These samples were analyzed for numbers of *Campylobacter* spp. Neck skins were also analyzed for *E.coli* (12-13 samples in each round). Carcass rinse samples were included to be able to compare results from neck skin and carcass rinse. Analysis of neck skin and caracass rinse was performed on the same carcass. The neck skin was removed prior to carcass rinse.

# **Microbiological methods**

<u>Skin samples:</u> An amount of 10 g neck skin was diluted 1:10 in Buffered Peptone Water (BPW), further serially diluted in BPW. Appropriate dilutions were plated on mCCDA-plates. For the *E. coli* analysis, the same dilution series was used, and 1 ml of the appropriate dilutions was plated on Petrifilm Select *E. coli* and incubated as described from the manufacturer.

<u>Carcass rinse</u>: One carcass was thoroughly rinsed in 200 ml BPW (as described in Boysen & Rosenquist, 2009), followed by centrifugation. The supernatant was removed and the pellet was resuspended in 10 ml BPW. Further serial dilutions were carried out and appropriate dilutions were plated on mCCDA.

<u>Caeca</u>: The content of one ceacum was "squeezed" out and diluted 1:10 in BPW, followed by further serial dilution and plating out of appropriate dilutions on mCCDA.

All mCCDA plates were incubated at 41.5°C for 48±4 hrs as described in NMKL no. 119, 2007. A total of five typical colonies from each positive sample were confirmed by colony morphology on blood agar and motility check by microscopy.

# Campylobacter results

An overview of the *Campylobacter* results is seen in table 3.

The data set includes censored data: there were some problems finding the correct dilution series for caeca, and neck skin and carcass concentrations were sometimes too low. In table 3 only the quantified samples are included. This has an impact on the yellow rows: some samples give < or > results, and are not included, although they would influence the results.

The data can be fitted to a distribution with a fitting method for censored data (MLE, assuming a normal distribution of the logs), but in many cases the number of non-censored observations is so low, that this does not make sense. Results for the data sets, where this fitting approach was feasible, are given in table 4. The results allowed us to estimate the between flock mean and standard deviation (sd).

Table 3. Observed mean and standard deviation (sd) of log cfu per g or per carcass, quantitative Campylobacter data. Yellow colored cells contain censored data. The mean and sd in the lower rows are mean and sd for the data in the columns.

٨	neck	ad		carcass			саеса			
A	mean	sa o ra	n D	4 2 2	0.70	-				
1	3.05	0.58	25	4.32	0.78	5	7.07	0.62	25	25 X > 5
2	2.63	0.57	25	4.66	0.35	5	7.97	0.62	25	
3	1.30	0.44	5	2.67	0.54	4	6.63	1.35	15	10x < 5
4	1.10	0.17	3	2.36	0.39	3	4.74	2.32	22	3x < 1
5	2.77	0.59	25	5.24	0.77	5	8.14	0.51	25	
В										
1	2.85	0.76	25	4.47	0.84	5	7.60	0.11	2	23x > 7.7
2	1.62	0.42	23	3.59	1.07	5	7.72	0.87	25	
3	2.91	0.51	25	4.48	0.45	5	8.09	0.53	25	
4	2.21	0.59	25	3.92	0.36	5	7.29	0.78	25	
5	2.53	0.50	25	3.74	0.55	5	7.87	0.56	25	
mean	2.23	0.51		3.91	0.65		7.38	0.85		
sd	0.71	0.15		0.86	0.27		1.02	0.61		

Table 4. Mean and sd of log cfu per g or per carcass, fitted for censored data. c indicates that mean and sd of the flock are estimated by MLE for censored data. Mean and sd in the lower row are the mean and sd of the means (obtained by an MLE for censored data) and the mean of the sds (if available).

	nock			carcass			(29(2		
۸	moon	cd	n	carcass			Caeca		
A 1		SU O FO	11 25	4 2 2	0.70	F	. Г		
1	3.05	0.58	25	4.32	0.78	5	>5		
2	2.63	0.57	25	4.66	0.35	5	7.97	0.62	25
3	<1			2.46	0.59	С	5.42	1.92	С
4	<1			2.01	0.17	С	4.13	2.72	С
5	2.77	0.59	25	5.24	0.77	5	8.14	0.51	25
В									
1	2.85	0.76	25	4.47	0.84	5	>7.7		
2	1.55	0.46	С	3.59	1.07	5	7.72	0.87	25
3	2.91	0.51	25	4.48	0.45	5	8.09	0.53	25
4	2.21	0.59	25	3.92	0.36	5	7.29	0.78	25
5	2.53	0.50	25	3.74	0.55	5	7.87	0.56	25
mean	2.16	0.57		3.89	0.59		7.28	1.06	
sd	0.91			0.95			1.41		

# E. coli results

TUDIE J	. 0030		HELK SKIII UU	tu (log tiu/g), ui	i were quantified, no censoring needed.
А		mean	sd	n	
	1	3.33	0.31	13	
	2	3.53	0.69	13	
	3	3.28	0.38	13	
	4	2.84	0.28	13	
	5	3.31	0.43	12	
В					
	1	3.79	0.39	13	
	2	3.35	0.69	13	
	3	3.43	0.38	13	
	4	3.30	0.45	12	
	5	3.64	0.28	12	

Table 5 Observed F colineck skin data (log cfu/g) all were quantified no censoring needed

Neck skins were also analyzed for *E.coli*. The results are shown in table 5.

Apparently there is not a lot of difference between the flocks.

# Derive distributions for caeca and neck skins within and between flocks

### Caeca

We have data for within and between flocks for the caeca. Two flocks do not give any data: they are all censored. For the other eight flocks, two contain censored data. But we can deal with that. By using the censored data MLE for within flock distributions, we get the following results (table 6 and table 7).

Table 6. Mean and sd for the 8 flocks by using censored data MLE for within flock distributions, caeca

mean	7.97	7.72	5.42	4.13	8.09	7.29	8.14	7.87	
st dev	0.62	0.87	1.92	2.72	0.53	0.78	0.51	0.56	
n	25	25	С	С	25	25	25	25	

Table 7. Mean and sd. First row is the estimated mean of the mean and sd for data without and including censored data for between flock means; the second row indicates the standard deviation of the mean (between flocks) and of the sds.

of the me	an (bettiet		and of the se	
	mean	sd	incl cens flo	cks
mean	7.08	1.48	7.28	1.41
Sd	1.06	0.81		

The difference with Table 4 is that we there included the censored data of the other two flocks for the mean.

Plotting the mean and the sd (fig. 1) illustrates that there is a correlation between the mean and the sd for the different flocks.

Figure 1. Mean and sd of numbers of Campylobacter, caeca



#### Neck skins

For the necks skins the results are shown in table 8 and fig 2.

Table 8. Mean and sd, neck skin

	neck skins	5							
mean	3.05	2.63	2.77	2.85	1.55	2.91	2.21	2.53	
sd	0.58	0.57	0.59	0.76	0.46	0.51	0.59	0.5	
	mean	sd	incl cens flo	ocks					
mean	2.56	0.48	2.16	0.91					
Sd	0.57	0.09							

Figure 2. Mean and sd of numbers of Campylobacter, neck skin



It is interesting that the mean of the sd (the mean within flock sd) is smaller than the sd of the mean (the between flock sd) for caeca, but not for neck skins. This is opposite to what we assumed previously (e.g. Nauta et al. 2012). However, if we include the censored data, the sd of the mean increases and we see that all between flock sds are larger than the within flock.

### **Flock analyses**

#### caeca - neck skin

The relation caeca- neck skin, for those flocks were we had sufficient data (table 3) is seen in fig. 3. The relation carcass data-neck skin data appears in fig 4.



Figure 3. Relation between caeca data and neck skin data

The range of values for concentrations in the caeca is very small, as we could not include the flocks where we did not have sufficient data. We also did a regression for censored data (see appendix 2) (using table 4), which finds a best model y = 1.58 x - 9.95 with standard deviation 0.39. Note that the two regression lines are very different.

The method used is disputable and the fit is weak, so we decided not to use this result in the risk assessment of CamCon WP4.



Figure 4. Relation carcass data and neck skin data

However, the data in fig. 4 are of interest if you want to compare carcass rinse data with neck skin data. It is previously assumed that the carcass has 100 g skin, so there is two logs difference (e.g. Nauta et al. 2013). However, the means differ 1.7 - 1.9 logs in Tables 3-5.

If a censored data regression is performed, the best fitting line is  $y=0.87 \times -1.25$ . In "Theory" this would be y = x - 2

The plot in fig. 5 shows the different data and the different lines through them.

Figure 5. Data points and different regression lines. Theory shows the two log difference; MLE fit the fit with regression for censored data, see appendix 2 (white squares indicate censored data); lin reg counts is the regression based on count data only.



#### **Individual samples**

If the neck skin data of *E.coli* and *Campylobacter* for the individual samples are plotted (fig. 6), there is a trend, but it does not look particularly convincing. There is a significant correlation though, if the MLE regression line is compared with no correlation at all. We performed both the regression analysis through the count data, and one using an MLE for censored data. (see appendix 2). This gave a *Campylobacter* concentration = 1.04 *E. coli* concentration – 1.29 with sd 0.9. Censored data analysis gave fits for normal distributions of the log concentrations: *E.coli* Normal(3.38, 0.49) and *Campylobacter* Normal(2.23, 1.05)



Figure 6. Relation log counts of E. coli - Campylobacter

A regression for neck skin data and carcass data was done for individual count data as well. The censored data are a problem again, they complicate the regression analysis. If we apply the Limit of detection as substitute for missing data, we get the regression line indicated in fig 7. We were not able to do a censored data regression in this case, because it is unclear how to include data pairs where both data are censored. It is likely that the slope of the line would increase, closer to the theoretical prediction.





#### **Conclusions for CamCon WP4**

#### **Regression model**

It would be interesting to derive a regression model for caecal counts and neck skin counts, as this could be used to assess the impact of reducing the caecal concentration. However, the size of the data set is limited, censored data complicate the analysis and the range of values for the caecal concentrations, where count data are available, is small. Therefore, the resulting model is not considered credible.

### Useful input data

We need the distributions of caecal concentrations in Norway for the analyses. For caecal concentrations we get the results in table 9 and the interesting correlation between mean and sd per flock in fig. 8.

Table 9. I	Vlean and s	sa, Campylo	bacter in	саеса
	mean	sd between	incl cen	s flocks
mean	7.08	1.48	7.28	1.41
sd within	1.06	0.81		

0 14

Figure 8. Correlation between mean and sd per flock



### Other general conclusions

- As seen previously, trends and correlations are much influenced by the high variability in the data.
- Censored data complicate the analyses.
- The data are useful to relate neck skin data with carcass rinse data. The means differ 1.7 1.9 logs, so less than the previously assumed 2 log.
- *E.coli* and *Campylobacter* do not correlate in a useful way.

# 3. Campylobacter in broiler caeca in the UK

Nicola Williams and Yvette Merga, University of Liverpool, UK (microbiological data) Maarten Nauta, Technical University of Denmark (data analysis)

The data presented in this section are *Campylobacter* enumeration data on 10 individual caeca per broiler flock of 13 contaminated flocks, obtained in the 20 farms study in CamCon WP1. The data were provided by the partner in the UK Caeca were sampled at slaughter from the flock batch undergoing thinning (partial depopulation). Contaminated caecal contents were enumerated by serial dilution and spread plating on modified charcoal-cefoperazone-deoxycholate agar (LabM, Bury UK) based on ISO 10272-2:2006 (ISO 2006), with a subset of isolates confirmed as *Campylobacter* species using a genus specific PCR assay (Katzav et al., 2008) The data were obtained in the fall/winter 2011/2012.

Table 10 gives the mean and standard deviation (sd) of the 13 flocks. All samples were counted, there are no censored data. Negative flocks and pooled sample data are not included.

flock	mean	sd	
1	8.24	0.73	
2	8.23	0.70	
3	8.01	0.87	
4	7.97	0.88	
5	7.85	0.56	
6	7.83	0.31	
7	7.76	0.48	
8	7.00	1.12	
9	6.92	0.67	
10	6.20	0.75	
11	6.04	0.55	
12	5.88	1.30	
13	5.39	1.53	

Table 10. Campylobacter mean and sd for 13 broiler flocks

As a result we obtain the mean and sd of the means per flock, and the mean and sd of the sds: 7.18, 1.00, 0.80 and 0.34, respectively. These data are used in the risk assessment in WP4.

Interestingly, the mean and sd per flock are correlated (fig. 9)



*Figure 9. Correlation between mean and sd per flock* 

<sup>[19]</sup> 

# 4. Interpretation of *Campylobacter* data from pooled caecal samples, Spain

Laura Laureano Adame, Nutreco Food Research Center, Spain (microbiological data) Maarten Nauta, Technical University of Denmark (data analysis)

Laureano et al. (2013) studied the correlation of different matrices with *Campylobacter* counts in neck skin of broiler carcasses. Among others, they obtained pooled samples of caeca of birds from 80 flocks from different processing plants in Spain. These pooled samples contained five samples per pool, and two pooled samples were obtained per flock. The data were kindly provided for further analysis in CamCon.

# **Microbiological method**

A total of 80 batches were analyzed, 10 per slaughterhouse, in order to evaluate the correlation between *Campylobacter* counts in caeca and neck skin of each batch. For *Campylobacter* enumeration in caeca, 10 broiler carcasses were randomly chosen from each batch. The sampling was done after bleeding step and before entering the scalder. The pair of caeca from each carcass was aseptically taken out and 2 pools (with 5 pairs of caeca per pool) were obtained. Summarizing, 2 samples were obtained from each batch. The total number of samples was 160.

Every 5 pairs of caeca, aseptically obtained, were placed into a sterile bag. This pool conformed one sample. Every sample was aseptically smashed to release the caeca content inside the bag. After this, the Buffered Peptone Water (BPW) volume needed to make 1:10 dilution was added using an automatic dilutor. Then each sample was homogenized with a peristaltic homogenizer (stomacher) for 30 seconds. After that, the samples were left at room temperature for 30 minutes.

Once we had the samples prepared the analysis was started by preparing serial decimal dilutions of each sample, by adding 1ml of 1:10 dilution to 9ml tube of BPW. The same step was repeated up to 1:1000000 dilution. After that, the samples were spread over the plates using sterile spreaders (Drigalski spatulas). The agar plates used were chromogenic medium *Campylobacter* Selective Agar (CASA<sup>®</sup>). The 1:10 dilution was spread using 3 plates (333µl per plate), the following dilutions were made by adding 100µl of the previous dilution in only one plate.

Once the plates were prepared, they were incubated for 48 h at 41.5 °C under microaerophilic conditions using GENbag microaer bags (Biomerieux). After the incubation, the plates were removed from the incubator and the *Campylobacter* colonies were counted. Suspicious *Campylobacter* colonies were confirmed by agglutination test using *Campylobacter* Latex Kit (Microgen) and dark field microscopic observation (morphology and mobility). Finally, the count obtained in each plate was multiplied by the dilution factor, to express the result as cfu per gram.

### Data analysis

In total 160 pooled samples of caeca were enumerated. The mean concentration found was 6.83 log cfu/g with a standard deviation of 1.27 (median 7.06). Of the 80 paired pooled samples, the mean concentration found was 6.83 log cfu/g with a standard deviation of 1.17 (median 7.03). The absolute value of the difference of the two pooled samples per flock had a mean of 0.63 log cfu/g with a standard deviation of 0.83 (median 0.34).

For the risk assessment in WP4 of CamCon (Nauta et al. 2015), mean and standard deviations of concentrations in the caeca of individual birds are required. The fact that the samples are pooled

complicates the interpretation of the data, as concentrations found in pools are expected to be larger as in individual samples (Bahrndorf et al. 2014). How much larger they are, depends on the distribution of concentrations in the individual samples. Still, the difference in concentration between the two pooled samples should somehow reflect the estimate of standard deviation between the concentrations in individual samples. Assuming a lognormal distribution of individual enumeration data, it is not possible to derive the mean and standard deviation of individual samples analytically, as, to our knowledge, there is no algebraic solution available to derive the mean and standard deviation of a sum of samples taken from a lognormal distribution.

Here we therefore used an approach where estimates for the required parameters for the distributions of concentration were obtained by computer simulation. Assuming Normal distributions of log concentrations, the expected values of

(1) the overall mean concentration in pooled samples,

(2) the <u>mean</u> absolute value of the <u>difference</u> in concentration between two pooled samples from one flock,

(3) the standard deviation of the means of two pooled samples per flock and

(4) the standard deviation of the absolute value of the differences between two flocks

were obtained by simulating the pooling process *in silico* by Monte Carlo simulations using @Risk software, assuming distributions of individual samples defined by m, s<sub>b</sub> and s<sub>w</sub>, with

m: the mean of the mean concentrations per flock.

 $s_b$ : the standard deviation of the mean concentration per flock (the between flock sd), so that the means per flock  $m_f$  (in log cfu/g) follow a Normal (m,  $s_b$ ) distribution.  $s_w$ : the standard deviation of concentrations (in log cfu/g) within flocks.

So 2x 5 samples were taken from a Normal( $m_f$ ,  $s_w$ ) distribution, the five samples were summed (as cfu/g, so after transformation) and divided by five, before re-transformation to log cfu/g, yielding a mean and a difference between concentrations found in the two pooled samples.

The question was what individual distributions of concentrations (in terms of m,  $s_b$  ad  $s_w$ ) were required to get results similar to the data. The best fitting values (obtained by calculating the least sum of squares of the difference in observed and simulated values of (1) - (4)) were selected after a trial and error process, using interpolation.

It was found that the "best fit" for all caecal count data is m = 6.0;  $s_b$  = 1.05;  $s_w$  = 1.17.

which for pooled data gives the results in table 11.

Table 11 Descriptive statistics of the log concentrations found in pooled samples obtained from the computer simulations and in the microbiological data. Given are the mean of means per two pooled samples per flock, the mean difference between the two pooled samples, the standard deviation of the means per two pooled samples per flock and the standard deviation of the difference between the two pooled samples

				e
		mean	sd	sd
	mean	difference	means	difference
simulation results	6.82	0.80	1.16	0.61
data	6.83	0.63	1.17	0.83

Visual inspection showed that the data contains four outliers with a large impact on the results. Here the difference between the two pooled samples is high, definitely compared to the mean of the two observed values.

Fig. 10 shows the adapted data set (left) and the original (right).

Figure 10 Adapted data set (left) and the original (right). The x axis shows the mean concentration in the pooled samples, the y-axis shows the difference between the two pooled samples.



Without the outliers it was found that the "best fit" for caeca is

 $m = 6.55; s_b = 0.85; s_w = 0.8.$ 

which for pooled data gives the values in table 12.

		mean	sd	sd
	mean	difference	means	difference
simulation results	7.01	0.52	0.91	0.40
data	6.99	0.48	0.90	0.47

Table 12 Descriptive statistics as in table 11, without the four outliers

This last result is applied in Nauta et al. 2015.

# 5. Campylobacter in Poland

Jacek Osek and Kinga Wieczorek, National Veterinary Research Institute, Poland

The following data (tables 13-15) were obtained from the partner in Poland. The flock data were obtained in WP1 and WP3 of the CamCon project and the meat data were obtained through the national monitoring program in Poland.

Farm	No.	Batch size of	Positive	s/samples	Swabs			
	sampled	slaughtered	ta	ken				
	flocks	broilers	Caeca	Carcass	Ca	ieca	Car	cass
	from				<b>0</b> //	<u> </u>	<b>0</b> //	<u></u>
	farm				C. coli	C. jejuni	C. coli	C. jejuni
1	11	3780-19700	7/11	7/11	1	6	1	6
2	7	7178-21097	6/7	3/7	2	4	0	3
3	10	5824-29050	10/10	5/10	4	6	2	3
4	12	5040-30000	12/12	9/12	6	6	3	6
5	5	2500-8500	3/5	4/5	1	2	1	3
6	8	16883-35886	8/8	5/8	6	2	1	4
7	7	15308-18200	7/7	4/7	6	1	2	2
8	5	9497-26549	3/5	5/5	1	2	2	3
9	4	4508-10633	3/4	2/4	0	3	0	2
10	6	4544-20000	4/6	2/6	0	4	0	2
11	5	4800-7488	3/5	2/5	2	1	0	2
12	10	4340-18000	10/10	6/10	4	6	1	5
13	10	4200-19488	6/10	5/10	2	4	0	5
14	9	3840-22330	7/9	5/9	1	6	1	4
15	7	5632-12000	5/7	1/7	0	5	0	1
16	9	9520-28000	7/9	5/9	1	6	2	3
17	7	5000-23000	7/7	6/7	1	6	1	5
18	8	15700-20350	8/8	4/8	2	6	1	3
19	10	4000-4000	9/10	6/10	1	8	0	6
20	7	3500-17000	3/7	4/7	2	1	1	3
21	4	70-320	3/4	3/4	1	2	1	2
22	9	4302-19768	3/9	4/9	1	2	1	3
23	11	4983-18229	10/11	7/11	3	7	1	6
24	2	4480-5376	2/2	1/2	1	1	1	0
25	7	7560-45500	7/7	6/7	0	7	0	6
26	8	3100-15000	6/8	1/8	3	3	0	1
27	7	3100-20300	5/7	1/7	2	3	0	1
28	9	2500-12000	9/9	9/9	7	2	5	4
29	11	4700-10000	11/11	11/11	9	2	6	5
30	10	4700-5600	10/10	10/10	9	1	6	4

Table 13. Campylobacter in caecal content and on carcasses for 30 farms, CamCon WP1.

Table 14. Results of swab samples taken from caecal contents and carcasses at slaughter for three flocks, CamCon WP3.

Flock	Date of	Age of	PCR identification			
number	slaughter	broilers	Caecal contens	Carcasses		
		(days)				
1	2012.08.28	56	C. coli	C. jejuni		
2	2012.08.21	56	C. coli	C. coli		
3	2012.10.26	49	C. coli	C. coli		

Table 15. Prevalence and number of Campylobacter species isolated from retail meat samples. April 2009-December 2012

Sample type	Number of samples tested /	Number po	Number (%) of samples positive for:		Number (%) of samples with <i>Campylobacter</i> spp. (cfu/g)			
	Number of Campylobacter - positive samples	C. coli	C. jejuni	<10 <sup>2</sup>	10 <sup>2</sup> -10 <sup>3</sup>	10 <sup>3</sup> -10 <sup>4</sup>	>104	
Chicken meat								
Wings	91/80	50 (62.5)	30 (37.5)	79 (86.8)	9 (9.9)	3 (3.3)		
Legs	239/202	113 (55.9)	89 (44.1)	187 (78.2	24 (10)	26 (10.9)	2 (0.9)	
Corpuses	57/49	26 (53.1)	23 (46.9)	47 (82.5)	3 (5.3)	6 (10.5)	1 (1.7)	
Filets	101/82	52 (63.4)	30 (36.6)	97 (96)	2 (2)	2 (2)		
Total	488/413	241 (58.3)	172 (41.7)	410 (84)	38 (7.8)	37 (7.6)	3 (0.6)	

Since the risk assessment uses concentration data from caecal samples and skin samples only, these data were not applied in the analyses in WP4.

# 6. The impact of thinning and slaughter age

Maarten Nauta, Technical University of Denmark

The aim of the analyses described in this section was to estimate the impact of a ban on thinning and a reduction of slaughter age. The impact of these interventions could not be assessed on the basis of the risk factor study in CamCon WP1. As the impact of thinning was believed to be substantial, data in WP1 are collected at first depopulation; thinning (= partial depopulation) is not included as a factor. And since slaughter age was not included in the questionnaire performed in CamCon WP1 (Høg et al. 2011), it is not possible to relate flock contamination with slaughter age in the data set.

As an alternative we apply the outcomes of the logistic mixed effect model based on the EU baseline survey data (EFSA 2010), which is also used in the EFSA opinion on *Campylobacter* control (EFSA 2011). As the models applied in the opinion are not given in detail, and the analysis is not performed for the six countries studied in CamCon, these analyses are included in this report. The reason for analysing the impact of these interventions is predominantly to allow us to include them in the cost effectiveness analysis (Van Wagenberg et al. 2015). The objective is to estimate the expected change in prevalence in the six CamCon countries due to a ban on thinning and a reduction of the slaughter age to maximum 35 days in all flocks.

For our analysis, we use data from EFSA (2010, 2011). The only specific CamCon data applied are those on the number of days between first and last depopulation.

# Model

The logistic regression model used in the model applied by EFSA (2011) and is given as

$$ln\frac{p}{1-p} = k_{MS} + \beta_1 * age_{MS} + \beta_2 * thin$$

with p the *Campylobacter* flock prevalence (entrance slaughter plant),  $k_{MS}$  a country specific constant,  $\beta_1$  and  $\beta_2$  coefficients as explained below,  $age_{MS}$  is the (mean) age at slaughter in the country and *thin* the status of thinning (1 = yes, 0 = no).

 $\beta_1$  and  $\beta_2$  are obtained from the statistical analysis of the EU baseline study 2008 (EFSA 2010) and are derived from the Odds Ratios found in the multivariate analysis performed. ( $\beta$  = ln(OR)). Here we use the OR estimates for age of slaughter and thinning, for countries with below median prevalence (NO, DK, NL) and for countries above median prevalence (ES, PL, UK) (table 16).

prevulence (NO, DK, NL) und jor countries above me						
	below m	above median				
	OR	β	OR	β		
thinning	1.49	0.399	2.12	0.751		
age	1.58	0.046	2.08	0.073		

Table 16. OR estimates for age of slaughter and thinning, for countries with below median prevalence (NO, DK, NL) and for countries above median prevalence (ES, PL, UK).

Note that the OR for age is based on ten days, the  $\beta_1$  value is expressed per day. Also note that the ORs are assumed to be the same for the grouped countries.

Our aim is to analyse the effects of thinning and slaughter age reduction separately. However, slaughter age reduction is a likely side effect of thinning (EFSA, 2011): the farmhouse is filled up with broilers at an earlier stage, so earlier depopulation is required to prevent overcrowding.

# Thinning

For thinning we ignore the difference between indoor and outdoor farms (EFSA 2011), because the percentage of farms categorized as "outdoor" is low in all six countries, and because insufficient data are available to do the analysis properly.

First, the impact of thinning on slaughter age is ignored. The *age* need not be included in the analysis.

The constant  $k_{\text{MS}}$  is derived from the data on the current situation. With thinning the Campylobacter flock prevalence

 $p_t = \frac{e^{k_{MS} + \beta_2}}{1 + e^{k_{MS} + \beta_2}}$ without thinning  $p_{not} = \frac{e^{k_{MS}}}{1 + e^{k_{MS}}}$ 

And the *Campylobacter* flock prevalence in the country is  $p = P(thin) * p_t + (1 - P(thin)) * p_{not}$ with P(thin) the probability of thinning. After a ban on thinning the prevalence becomes  $p_{not}$ .

Using the published *Campylobacter* prevalence, mean slaughter age and thinning frequency per country (EFSA 2010), the value of  $k_{MS}$  can be obtained, e.g. by using the Excel Solver function.

Results are given in table 17.

Table 17. Country specific values for the frequency of thinning (P(thin)), the prevalence of infected flocks (p), the flock prevalence after a ban on thinning ( $p_{not}$ ) and the relative risk reduction after a ban on thinning, without taking the slaughter age into account (rel red = 1- $p_{not}/p$ ),

<u></u> ,,,,,,,		e era agricer	age mee e	
	P(thin)	р	<b>p</b> <sub>not</sub>	rel red
Denmark	24.8%	19.2%	17.6%	8.4%
Poland	49.4%	79.2%	73.3%	7.5%
Spain	49.1%	87.7%	83.8%	4.4%
The Netherlands	42.2%	24.2%	21.1%	12.9%
United Kingdom	63.4%	75.8%	66.8%	11.9%
Norway	3.3%	3.3%	3.2%	1.5%

Next, we can include the fact that a ban on thinning reduces the slaughter age.

From the questionnaire (Høg et al. 2011), CamCon provides data on the mean number of days between first and last depopulation  $t_{MS}$ . We ignore the fact that the same flock may be depopulated more than once.

The flocks that are not thinned have a slaughter age of  $t_{MS}$  more days than those that are thinned. As the mean slaughter age  $age_{MS}$  in a country

 $age_{MS} = age_{MS,t} * P(thin) + age_{MS,not} * (1 - P(thin))$ and  $age_{MS,t} = age_{MS,not} - t_{MS}$  it follows that  $age_{MS,not} = age_{MS} + P(thin) * t_{MS}$ and  $age_{MS,t} = age_{MS} - (1 - P(thin)) * t_{MS}$ 

So we find the new value of  $k_{MS}$ . With thinning  $e^{k_{MS}+\beta_2*\alpha_{ge_{MS,t}}+\beta_2}$ 

 $p_t = \frac{1}{1 + e^{k_{MS} + \beta_1 * \alpha_{gBMS,t} + \beta_2}}$ 

without thinning  $p_{not} = \frac{e^{k_{MS} + \beta_1 * \alpha_g e_{MS,not}}}{1 + e^{k_{MS} + \beta_1 * \alpha_g e_{MS,not}}}$ 

Knowing that the *Campylobacter* flock prevalence in the country is  $p = P(thin) * p_t + (1 - P(thin)) * p_{not}$ 

We can find  $k_{\text{MS}}$  and calculate  $p_{\text{not}}$ 

The results are given in table 18.

Table 18. Country specific values for the frequency of thinning (P(thin)), the mean slaughter age  $age_{MS}$ , the mean number of days between the first and last depopulation  $t_{MS}$ , the prevalence of infected flocks (p), the flock prevalence after a ban on thinning ( $p_{not}$ ) and the relative risk reduction after a ban on thinning, taking the reduction of slauh'ghter age into account(rel red =  $1-p_{not}/p$ ),

	P(thin)	age <sub>Ms</sub> (days)	t <sub>MS</sub> (days)	р	p <sub>not</sub>	rel red
Denmark	24.8%	37.81	5.30	19.2%	16.5%	13.9%
Poland	49.4%	44.32	7.10	79.2%	69.6%	12.2%
Spain	49.1%	47.75	11.20	87.7%	80.4%	8.3%
The Netherlands	42.2%	41.14	7.70	24.2%	18.4%	24.2%
United Kingdom	63.4%	41.14	7.80	75.8%	60.0%	20.8%
Norway	3.3%	32.37	5.10	3.3%	3.2%	2.7%

Clearly, the impact of thinning increases if the reduction of slaughter age is taken into account.

### Slaughter age

We want to study the impact of reducing the slaughter age to maximum 35 days. Data we have per country are the mean and standard deviation of the slaughter age, plus a minimum and maximum, in the data from the EU baseline study (EFSA 2010). We need to know not only how many are <35 days now, but also what the distribution of slaughter ages is: the higher the age, the larger the probability of infection of the flock. This distribution will change: It is assumed here that all flocks > 35 days will become slaughtered at exactly 35 days. For convenience it is assumed that slaughter days in each country follow a normal distribution, with the mean and standard deviation given.

A new value for  $k_{MS}$  has to be found. If the prevalence at a certain slaughter age in a country is  $e^{k_{MS}+\beta_{1}*\alpha_{ge}}$ 

 $p_{age} = \frac{1}{1 + e^{k_{MS} + \beta_{1} * age}}$ 

and we know that the probability of slaughter age *age*, P(age), is obtained from the normal density function, in Excel this can be done by using the function NORMDIST(age, mean age, stdev age).

Then the current Campylobacter prevalence

$$p = \sum_{age=20}^{85} P(age) * p_{age}$$

 $k_{MS}$  is obtained from this equation.

After intervention the distribution of slaughter ages changes so that if

$$P'(x) = \sum_{age=x}^{85} P(age)$$

we want to obtain the value of P'(35), and after intervention

$$p' = \sum_{age=20}^{5^*} P(age) * p_{age} + P'(35) * p_{35}$$

The results appear in table 19.

Table 19. Country specific values for the % of flocks slaughtered within 35 days, the mean and standard deviation of the slaughter age  $age_{MS}$ , the prevalence of infected flocks (p), the flock prevalence after reduction of the slaughter age ( $p_t$ ) and the relative risk reduction after reduction of slaughter age(rel red = 1-p'/p).

	days <= 35	mean	st. dev.	n	n'	rol rod
	1-P'(36)	age <sub>MS</sub> (days)	age <sub>Ms</sub> (days)	μ	þ	Terreu
Denmark	15.0%	37.81	5.30	19.2%	17.2%	10.6%
Poland	2.6%	44.32	7.10	79.2%	66.5%	16.1%
Spain	5.4%	47.75	11.20	87.7%	75.5%	13.8%
The Netherlands	14.7%	41.14	7.70	24.2%	19.0%	21.6%
United Kingdom	19.8%	41.14	7.80	75.8%	66.9%	11.8%
Norway	94.9%	32.37	5.10	3.3%	3.3%	0.4%

#### **Results and discussion**

The estimated impact of banning thinning and reducing the slaughter age to 35 days is seen for six European countries in table 20 and fig. 11.

thinning						
	DK	ES	NL	NO	PL	UK
no thinning now	75.19%	50.90%	57.83%	96.72%	50.58%	36.62%
no thinning future	100%	100%	100%	100%	100%	100%
prev now	19.2%	87.7%	24.2%	3.3%	79.2%	75.8%
prev future	16.5%	80.4%	18.4%	3.2%	69.6%	60.0%
slaughter age						
	DK	ES	NL	NO	PL	UK
slaughter age < 35 now	15.0%	5.4%	14.7%	94.9%	2.6%	19.8%
slaughter age < 35 future	100%	100%	100%	100%	100%	100%
prev now	19.2%	87.7%	24.2%	3.3%	79.2%	75.8%
prev future	17.2%	75.5%	19.0%	3.3%	66.5%	66.9%

Table 20. The impact of thinning and slaughter age in six countries

Figure 11. Prevalence and % risk reduction in six European countries with a ban on thinning excluding (1) and including the effect on slaughter age (2) and with reduction of the slaughter age to 35 days.



These analyses are based on EFSA data (2010 and 2011), not on CamCon data. If the prevalences of thinning in EFSA (2011) and CamCon (Høg et al. 2011) are compared, you get the results in fig. 12.

*Figure 12. Comparison of prevalences of thinning based on EFSA (2011) and CamCon (Høg et al. 2011)* 



There clearly are some differences. As the model (Van Wagenberg et al. 2015.) is based on the EFSA prevalences, these are to be used further.

In comparison to what was done in the EFSA reports (2010, 2011) the effect of season is ignored and the effect of indoor/outdoor is ignored. It is not clear how that impacts the results.

EFSA 2011 analyses three of six countries in CamCon : NO, DK, UK.

For thinning, EFSA reports relative risk reductions of 1.8%, 12.6% and 25.7% for NO, DK and UK, compared to 2-3.2%, 8.5-14% and 11.9-20.8% estimated in this report. Differences in the models are seasonality, distinction indoor/outdoor, the fact that EFSA (2011) only uses one OR (no distinction high and low prevalent countries) and that it is not really clear from the report (EFSA 2011) how the calculations are actually done (especially inclusion of slaughter age). As the results are similar, it seems as if the two approaches are similar.

For slaughter age, EFSA states that the effect of a slaughter age reduction to 35 days gives 0.6 – 18% risk reduction for all EU countries. We find 0.4 – 22% in six countries. Again, it is not completely clear how the analyses in EFSA (2011) are done, but the results by EFSA and us are pretty similar.

The assumption that the  $\beta$  values are the same for different countries has impact: as the differences in slaughter age are small, the difference in prevalence between countries is predominantly determined by difference in k<sub>MS</sub> (the intercept of the regression line). For e.g. slaughter age to have a large impact, the slope ( $\beta$ ) should be different, but it cannot be, because we assume it is the same. Therefore, it is an advantage that we make a difference between high and low prevalent countries.

# Conclusion

Models provided by EFSA have been used to assess the impact of a ban on thinning (partial depopulation) and reduction in slaughter age to maximum 35 days on Campylobacter flock prevalence. The data used are those obtained by EFSA in 2008 (published *Campylobacter* prevalence, the mean and standard deviation of the slaughter age, and thinning frequency per country) and some data obtained in CamCon (the mean number of days between first and last depopulation). The results used in the study on the effects of interventions at primary production are shown in table 20.

# 7. The impact of fly screens

Helle M. Sommer, Technical University of Denmark

Similar to the section above estimating the impact of thinning and slaughter age, the impact of fly screens could not be estimated from CamCon WP2. Even though studies investigating the effect of fly screens in the UK and Spain were part of the CamCon project, the experiments were either inconclusive or ongoing when data for the risk assessment were needed.

We therefore decided to use published data from Denmark (Bahrndorff et al., 2013) as basis for estimation of the impact of fly screens on the broiler flock prevalence. In this study, fly screens were only installed on selected Danish broiler houses with a high level of bio-security. Therefore, the estimates of the impact of fly screens can only be applied to this kind of houses, i.e. Danish houses with a high level of biosecurity. From the CamCon risk factor study (Sommer et al., 2015) we found significant factors indicating that high biosecurity could be translated into farms with both anteroom and barriers in all houses, farms with dedicated tools for each house, and farms where the youngest house at the farm is < 15 years old. Data from the questionnaire in CamCon WP1 (Høg et al. 2011) and the risk factor study (Sommer et al., 2015) were used to calculate the percentage of houses in this category; 33 % (25+8) (table 21).

Table 21. Percentage of houses complying with the requirements; Danish, high biosecurity. Tools Yes and No to the question on dedicated tools for each house, Anteroom+barrier Yes and No to the question on both anteroom and barriers in all houses. Red numbers in bold are those complying with the requirements.

Poro	ontage of all bourses	Anteroom	cum	
Feic	entage of all nouses	Yes	No	Sum
Tools	Age of newest house			
	0-5 years	8	3	11
Yes	6-15 years	25	21	46
	> 15 years	17	16	33
	0-5 years	1	•	1
No	6-15 years		1	1
	> 15 years	4	4	9
	Sum	55	45	100

The impact of applying fly screens was thus only modelled for these Danish high biosecurity broiler houses. If fly screens were to be applied to all high biosecurity houses in the other 5 EU countries, then 28 % of all houses from the questionnaire in CamCon WP1 (Høg et al. 2011) would be selected. In the Danish fly screen studies (Hald et al., 2007; Bahrndorff et al., 2013) the screened houses belonged primarily to the age category 6-15 years. Three houses were older (in the category > 15 years old), but these had been renovated.

By combining the impact of fly screens from Bahrndorff et al. (2013) with the number of farms in the high biosecurity category, we can estimate the effect of applying the intervention 'fly screens'. The monthly prevalence values for screened, non-screened and control houses are seen in table 22 together with the national *Campylobacter* prevalence data (Bahrndorff et al. 2013). The prevalence values were estimated for two periods, 2003-2005 and 2006-2009. In the first period, the selected houses had fly screens and in the second period the selected houses were without fly screens. The right part of table 22 shows the estimated mean values for the figures in the left part of the table

for the 'summer-period' (July-November) and for 'winter-period' (December-June). An exception is the figures in green, bold, 9<sup>th</sup> column which are multiplication factors (explained below).

Table 22. Danish farm house data. Monthly prevalences for screened, non-screened, control houses, and national data (first part of table) and mean values for 'summer' and 'winter' prevalences (second part of table). Figures in green, bold in column nine are relative multiplication factors. Figures in yellow, bold, third column are used in calculating the multiplication factors and are explained in the text below.

	-nets	+nets	Control	Control	National	National	-nets	+nets	Control	Control	National	National
	2003-5	2006-9	2003-5	2006-9	2003-5	2006-9	2003-5	2006-9	2003-5	2006-9	2003-5	2006-9
Jan	4%	0%	5%	4%	20%	12%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
Feb	6%	7%	13%	4%	19%	14%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
Mar	0%	8%	0%	8%	19%	11%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
Apr	0%	0%	0%	8%	14%	12%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
May	0%	4%	0%	0%	14%	19%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
June	5%	10%	17%	12%	37%	28%	3.1%	5.3%	5.0%	5.1%	20.1%	16%
July	54%	20%	57%	52%	52%	54%	47.2%	3.78	41.4%	36%	46.2%	44%
Aug	47%	9%	56%	52%	64%	63%	47.2%	1.42	41.4%	36%	46.2%	44%
Sep	67%	7%	42%	44%	57%	47%	47.2%	1.42	41.4%	36%	46.2%	44%
Oct	25%	5%	33%	20%	33%	37%	47.2%	1.42	41.4%	36%	46.2%	44%
Nov	43%	<mark>9%</mark>	19%	12%	25%	19%	47.2%	1.42	41.4%	36%	46.2%	44%
Dec	7%	8%	0%	0%	18%	16%	3.1%	5.3%	5.0%	5.1%	20.1%	16%

The largest effect on the prevalence values for the screened houses is seen in the period from July to November. The figures in yellow, bold, third column (houses with nets) are considerable lower than the figures to the left (same houses without nets). In order to estimate the impact of fly screen on the Danish monthly prevalence data, the figures in blue, bold, second column in table 23 should be reduced to a lower prevalence estimate. The impact of fly screens can be estimated using different approaches.

### Two different ways of estimating the effect of applying fly screens

1) Direct comparison of the average summer levels – using the yellow figures in bold, third column in table 22.

Since the summer levels (2003-2005) of the control and the '-net' houses are about the same (47.7 % and 41.4 %) as the summer level for the national data (46.2 %) it can be argued that new screened Danish houses would reach the same levels as the '+net' houses, yellow figures in bold, third column in table 22. (They may even reach lower prevalence values since the national prevalence estimate (2010-2011) has gone even further down since 2006-2009).

However, one could also argue that the '-net' and control houses are different from the average national houses, since the winter periods are quite different from each other (3.1 and 5 % versus 20.1 %) and therefore the direct reduction on the yellow figures in bold, third column cannot be applied.

If this method using the yellow figures in bold, third column as an estimate for newly screened houses is applied to the EFSA-data for DK (EFSA 2010) (table 23), then the months August-November would reach lower prevalence values (9 %, 7 %, 5 %, 9 %) than the EFSA-winter months (Dec-June); 9.9 %, which seems wrong. The EFSA prevalence is higher in the winter period (9.9 %) compared with the estimate of newly screened Danish houses, which may argue

against using this method. Moreover, the EFSA data show a lower summer prevalence (32.5 %) compared to the netted- and control-houses and national data (43 %), which may argue for even lower prevalence values for the newly screened houses.

2) Relative comparison according to the average winter level – using the green figures in bold, column nine in table 22

The relative increase in the average winter level for the '+net' houses (5.3 %, table 22 last part) to the summer level for the same houses is calculated and given in table 22 last part in green, bold, 9<sup>th</sup> column. These multiplication factors  $f_i$  are given in the equation below. From the CamCon study, the monthly prevalence for farms with houses <15 years of age with high biosecurity level is given in table 23. The average winter level for these houses is 4 %. Multiplying the 4 % with the factors  $f_i$  given in green, bold, 9<sup>th</sup> column in table 22 results in the prevalence values  $P_{i,+net,Camcon}$  given in red bold, column 4 in table 23. These estimates prevalence values for screened houses are a bit lower than the values from method 1) given in yellow in table 22 in bold, third column.

$$f_i = P_{i,+net} / P_{j,mean}$$
, where  $P_{j,mean} = 5.3\%$   
 $P_{i,+net,Camcon} = P_{j,mean,Camcon} \cdot f_i$ , where  $P_{j,mean,Camcon} = 4\%$ 

where *P* is a prevalence and *i* is an index for the summer months July to November and *j* is an index for the winter months December-June.

estimat	ea prevalence v	aiues for screer	nea nouses in	the Camcon st	uay using met	.noa 2).
	CamCon,	CamCon,	CamCon,	EFSA	EFSA	EFSA baseline
	-net,	-net,	+net,	all houses,	all houses,	+net,
	obs.	mean	estimate	obs.	mean	estimate
Jan	4%	4%		6.7%	9.9%	
Feb	2%	4%		11.4%	9.9%	
Mar	5%	4%		0%	9.9%	
Apr	0.5%	4%		8.6%	9.9%	
May	2%	4%		5.9%	9.9%	
June	12%	4%		22.6%	9.9%	
July	35%	20%	15%	43.8%	32.5%	31.5%
Aug	30%	20%	5%	51.6%	32.5%	11.8%
Sep	18%	20%	5%	22.2%	32.5%	11.8%
Oct	11%	20%	5%	26.7%	32.5%	11.8%
Nov	6%	20%	5%	18.2%	32.5%	11.8%
Dec	1%	4%		14.3%	9.9%	

Table 23.Danish farmhouse data. Columns stating –net and +net are selected houses with <15 years of age and with high biosecurity level. Red numbers in bold, column 4 and 7 are the estimated prevalence values for screened houses in the CamCon study using method 2).

When calculating directly from the tables 22 and 23 the following results are obtained from the two methods:

1) High-biosecurity houses are reduced from 10.6 % to 6.4 % (CamCon data).

10.6 = (4+2+5+0.5+2+12+35+30+18+11+6+1)/12

6.4 = (4+2+5+0.5+2+12+20+9+7+5+9+1.3)/12

Overall prevalence is reduced from 11.2 % to 9.5 % (CamCon data)

Overall prevalence of 11.2 is from section 8 in this report.

9.5 = 0.33\*6.4 + 0.66\*11.2

(33% of all Danish houses are high biosecurity as defined above and are here applied with fly screens).

Overall prevalence is reduced from 19.3 % to 15.7 % (EFSA 2010)
Overall EFSA prevalence of 19.3 for DK is given EFSA (2010).
Estimate of screened houses with high biosecurity for the EFSA data:
((9.9-1.6)\*7+20+9+7+5+9)/12 = 9.1
(the winter prevalence of 9.9 is reduced by 1.6 since the houses are high biosecurity houses; the 1.6 was observed in the CamCon data between the prevalence for all houses and high biosecurity houses).
15.7 = 0.33\*9.1 + 0.66\*19.3
2) High-biosecurity houses are reduced from 10.6 % to 5.3% (CamCon data)
5.3 = (4+2+5+0.5+2+12+15+5+5+5+1)/12
Overall prevalence is reduced from 11.2% to 9.1 % (CamCon data)
9.1 = 0.33\*5.3 + 0.66\*11.2
Overall prevalence is reduced from 19.3 % to 16.5 % (EFSA 2010)
Estimate of screened houses with high biosecurity for the EFSA data:

11.4 = ((9.9-1.6)\*7+31+12+12+12+12)/12

16.5 = 0.33\*11.4 + 0.66\*19.3

(Due to rounding off in the equations above the figures may not fully add up.)

The estimate of the effect of applying 'fly screens for selected houses' (33 %) differs depending on the two methods described above. For the EFSA data (EFSA 2010) method 1) resulted in an overall prevalence of 15.7 %, whereas method 2) resulted in an overall prevalence of 16.5 %. We chose to use method 2) for two reasons. First of all because it is related relatively to the winter period for the same houses and secondly because we wished to choose the most conservative estimate – not promising too much in the light of the many uncertainties and assumptions that goes with these calculations.

Running the risk factor analysis with the input from the calculated figures above from method 2) resulted in an overall prevalence reduction from 19.3 % to 16.6 % (EFSA level). The result 16.6 % was used in the cost-benefit analysis in CamCon WP4.

# 8. Campylobacter prevalences in broiler flocks

Birgitte Borck Høg, Technical University of Denmark (data analysis) Merete Hofshagen and Bruce David, Norwegian Veterinary Institute, Norway (data) Jaap Wagenaar, LEI Wageningen University and Research Center, the Netherlands (data) Jacek Osek and Kinga Wieczorek, National Veterinary Research Institute, Poland (data) Marta Cerdà Cuéllar, Campus Universitat Autònoma de Barcelona (data) Nicola Williams and Yvette Merga, University of Liverpool, UK (data)

*Campylobacter* prevalences of broiler flocks were collected in CamCon from the Netherlands, Poland, Spain and the UK. The data were collected for different purposes among others the risk factor study in WP1.

From Norway and Denmark, we used retrospective data from the existing national surveillance programmes for *Campylobacter*. From the Netherlands, Poland, Spain and the United Kingdom, data were collected from the longitudinal study of flocks on 20 farms described in WP1. Data from more than 6000 broiler flocks were collected and the occurrence of *Campylobacter* over the year in the participating countries is shown in Figure 13. There were significant differences between the participating countries.



Figure 13. Percentage of Campylobacter positive flocks in six European countries

In Norway, data was collected for the years 2010, 2011 and 2012. Due to the very low prevalence of *Campylobacter* in Norwegian broiler flocks during the winter, samples are collected in only from flocks slaughtered from May to October. The overall prevalence from flocks during this period was 3.7%. In Denmark, full annual datasets were obtained from 2010 and 2011. A clear seasonal trend was observed and the overall occurrence in the Danish broiler flocks was 11.2%.

From Poland, Spain and the United Kingdom, data were collected for the 20 farm study from 2011-2013, from 276, 201 and 219 flocks, respectively. In the Netherlands, data were collected from 221 flocks in 2012 and 2013. There was a clear seasonal distribution in Dutch data, with less than 20% positive flocks from November through February. The seasonal variation was less pronounced in Poland, Spain and the United Kingdom, where the percentage of positive flocks was never below 59%, 26% and 29%, respectively. No farms from the 20 farm study were consistently negative throughout the study period. However, almost a third of the participating farms in Poland were tested consistently positive during the study period.

# 9. Data on costs

Peter van Horne, LEI Wageningen University and Research Center, the Netherlands Coen van Wagenberg, LEI Wageningen University and Research Center, the Netherlands

Data were collected to estimate the costs of *Campylobacter* interventions on broiler farms in six European countries, Denmark, the Netherlands, Norway, Poland, Spain and the UK.

#### Technical and economic farm performance data per country

Basic data were collected in the period April to august 2011. Data on the year 2009 were collected if available, and otherwise as close as possible. The results are seen in Tables 24-26. The survey was performed by project partners for each participating country. These were:

Denmark:	Birgitte Borck Høg: <u>bibo@food.dtu.dk</u> DTU institute In cooperation with: the Danish Agriculture and Food Council
NL:	Peter van Horne: <u>peter.vanhorne@wur.nl</u> LEI Wageningen UR Based on FADN / BIN data
UK:	Nicola Williams: <u>njwillms@liverpool.ac.uk</u> University of Liverpool In cooperation with the industry and ADAS
Poland:	Pawel Kuayk: <u>pawel.kusyk@piwet.pulawy.pl</u> National Veterinary Research Institute
Norway:	Bruce David: <u>bruce.david@vetinst.no</u> Veterinarian Institute
Spain:	Marta Cerdà Cuéllar: <u>marta.cerda@cresa.uab.cat</u> Campus Universitat Autònoma de Barcelona In cooperation with the industry

Prices were converted into euro based on average exchange rate of 2009. Exchange rates, average for 2009 used in the calculations: Denmark: 0.1343 euro/DK krone Norway: 0.1146 euro/NO krone Poland: 0.2311 euro/zloty UK: 1.1224 euro/pound

Table 24 provides the general and financial data valid for all broiler farms in a country. Annual depreciation costs were assumed to be 4.0% of investment for housing and 8.0% for inventory, and annual maintenance costs 1.0 % of the investment for housing and 2.0% for inventory (Vermeij et al., 2009). Manure production was assumed to be 10.9 kg per broiler per year, water costs €0.80 per 100 broilers per cycle, and levies and dead animal removal costs €0.61 (Vermeij et al., 2009).

Table 24. General and financial data for Denmark (DK), Spain (ES), Netherlands (NL), Norway (NO), Poland (PL) and United Kingdom (UK). Year 2009, Prices and costs in local currency and euros.

All farms	DK	DK	ES	NL	NO	NO	PL	PL	UK	UK
General data										
Interest rate (%)		4.20%	5.00%	5.00%		4.00%		7.00%		5.50%
max density (chicken/m2)		38.0	42.0	42.0		36.0		42.0		38.0
Financial data										
Currency	DK-krone	Euro	Euro	Euro	NO-krone	Euro	Złoty	Euro	Pound	Euro
Labour cost per hour (incl. social tax) (€/hour)	175.00	23.50	14.00	21.33	189.00	21.66	10.00	2.31	15.00	16.84
Investment amount in house (cost/m2)	2,005.00	269.27	152.00	190.00	2,836.82	325.10	600.00	138.66	145.00	162.75
Investment amount in inventory (costs/m2)	703.00	94.41	64.00	84.00	1,254.18	143.73	225.00	52.00	62.00	69.59
Feed price (/100 kg feed)	205.00	27.53	27.00	26.22	391.28	44.84	113.10	26.14	23.80	26.71
Day old chick (costs/chick)	2.35	0.32	0.31	0.30	4.70	0.54	1.33	0.31	0.29	0.33
Manure disposal costs (costs/metric ton)		0.01	-5.00	16.00		0.01		-1.00		-3.00
Revenue price (revenue/kg live weight)		0.86	0.82	0.80		1.36		0.79		0.80
Overheads (€/farm)		11,600	9,280	11,600		11,600		8,120		11,600

Source: all variables from project partners, only max density from EU and national legislations.

Litter costs for farms that practice thinning were assumed to be €0.90 per 100 broilers per cycle (Vermeij et al., 2009). For all countries the percentage of broilers depopulated is 30% and the final delivery is the remaining 70%. Table 25 presents the other production and financial data for farms that practice thinning that differ between countries.

Table 25. Production and financial data for farms with depopulation (thinning)

Farms with thinning	DK	DK	ES	NL	NO	NO	PL	PL	UK	UK
Production data										
Growing period 1st delivery (days)		34.0	42.0	34.0		28.0		38.0		37.0
Growing period 2nd delivery (days)		40.0	48.0	42.0		33.0		43.0		42.0
Empty period (days)		8.0	15.0	8.0		19.0		18.0		7.0
Feed conversion in calculations		1.66	1.95	1.68		1.53		1.80		1.70
Stocking density house on day 1 (birds/m2)		22.5	18.3	25.0		26.0		21.3		19.8
Final live weight 1st delivery (g)		1,740	2,390	1,740		1,410		2,060		1,980
Final live weight 2nd delivery (g)		2,230	2,870	2,290		1,840		2,470		2,390
Mortality (%), on farm level		3.70%	4.50%	3.80%		3.20%		4.80%		3.50%
Financial data										
Currency	DK-krone	Euro	Euro	Euro	NO-krone	Euro	Złoty	Euro	Pound	Euro
Heating costs (costs/bird/cycle)	0.56	0.075	0.041	0.050	0.834	0.096		0.050	0.044	0.049
Veterinary costs (costs/bird/cycle)		0.040	0.032	0.040	0.018	0.002	0.190	0.044		0.040
External labour (€/bird/cycle)		0.050	0.030	0.045		0.046		0.005		0.036
Interest animals and feed (€/bird/cycle)		0.004	0.005	0.005		0.004		0.007		0.006
Electricity (€/bird/cycle)		0.016	0.022	0.025		0.014		0.018		0.020

Source: growing period and final live weight adapted from growing period and final live weight received from local project partner; empty period from Høg et al. (2011); feed conversion, mortality, heating costs and veterinary costs from local project partner; stocking density from national legislation (see Table 24); external labour costs Netherlands from Vermeij et al. (2009) and other countries adapted relative to wage level in country; interest costs Netherlands from Vermeij et al. (2009) and other countries adapted relative to interest level in country; Electricity costs Netherlands from Vermeij et al. (2009) and other countries adapted relative to electricity price level in country from Eurostat (variable nrg\_pc\_205, 20 MWh < Consumption < 500 MW, year 2009).

Many data were the same for farms with and without depopulation. Data for farms without depopulation that differ from farms with depopulation is presented in Table 26.

Table 26. Production and financial data for farms without depopulation (no thinning)

									·	·
Farms without thinning	DK	DK	ES	NL	NO	NO	PL	PL	UK	UK
Production data										
Growing period 1st delivery (days)		40.0	48.0	42.0		33.0		43.0		42.0
Feed conversion in calculations		1.72	2.01	1.75		1.58		1.85		1.75
Stocking density house on day 1 (birds/m2)		17.6	15.3	19.0		20.0		17.8		16.4
Final live weight 1st delivery (g)		2,230	2,870	2,290		1,840		2,470		2,390
Mortality (%), on farm level		3.81%	4.61%	3.94%		3.29%		4.89%		3.59%
Financial data										
Currency	DK-krone	Euro	Euro	Euro	NO-krone	Euro	Złoty	Euro	Pound	Euro
Heating costs (costs/bird/cycle)		0.096	0.049	0.066		0.124		0.060		0.060
Litter (€/bird/cycle)		0.008	0.007	0.008		0.008		0.007		0.007

Source: Growing period and final live weight equal to growing period 2<sup>nd</sup> delivery (see Table 25); Feed conversion calculated from feed conversion for a farm with depopulation with correction of 0.01 per 25 gram live weight; Stocking density is calculated based on national legislation (see table 24); Mortality calculated from mortality depopulation with correction 0.06% per day (2007/43/CE); Heating and litter costs per cycle do not depend on average stocking density, so adapted from situation with thinning to the lower average stocking density in situation without thinning.

#### Number of farms and broilers

The number of broilers per farm and the number of farms with broilers in each country were retrieved from Eurostat (Number of farms and heads by agricultural size of farm (UAA) and size of broiler flock [ef\_lsbroiaa] in 2010). The results are seen in Table 27.

Nationa	l broile	r population	S								
Poultry: Numb	per of farms	and heads by agricul	tural size o	f farm (UAA	A) and size o	of broiler flo	ck [ef_lsbro	iaa]			
		number of farms	number of	farms per	farm size						
GEO/N_HEAD	TIME	TOTAL	0	1-99	100-999	1000-2999	3000-4999	5000-9999	10000-49999	50000-99999	≥100000
Denmark	2010	280	0	110	0	0	0	10	50	70	40
Spain	2010	36570	0	32950	140	60	60	250	2500	470	140
Netherlands	2010	640	0	0	0	10	10	10	270	200	140
Poland	2010	337540	0	332930	2110	100	70	220	1640	300	170
United Kingdo	2010	1740	0	530	90	50	30	40	390	230	380
Norway	2010	430	0	50	0	0	10	30	270	50	20
Poultry: Numb	per of head	s (*1000) by agricultu	ral size of fa	arm (UAA) a	and size of	broiler flock	[ef_lsbroiaa	a]			
		number of broilers	number of	broilers pe	er farm size						
GEO/N_HEAD	TIME	TOTAL	0	1-99	100-999	1000-2999	3000-4999	5000-9999	10000-49999	50000-99999	≥100000
Denmark	2010	12840	0	0	0	0	0	40	1550	4730	6490
Spain	2010	118850	0	400	30	110	230	1860	59360	30540	26320
Netherlands	2010	44750	0	0	0	10	30	100	7810	14150	22650
Poland	2010	102180	0	4850	330	180	260	1690	37000	20190	37680
United Kingdo	2010	104180	0	10	30	80	100	320	10990	17160	75490
Norway	2010	11850	0	0	0	0	50	190	5650	3/190	2460

Table 27. National broiler population: number of farms and number of broilers

#### National broiler production and trade

*National broiler production, imports and exports* The data obtained are seen in Table 28.

Table 28. National broiler production and trade

· · ·	unit	DK	ES	NL	NO	PL	UK
gross domestic production broilers	(sl. weight in tons per year)	168,600	1,179,470	763,541	71,229	1,059,780	1,270,000
imported live broilers	(sl. weight in tons per year)	3,999	7,857	102,500	-	31,138	6,126
exported live broilers	(sl. weight in tons per year)	51,148	2,879	21,300	-	10,187	7,438
import of broiler meat and meat products	(sl. weight in tons per year)	47,678	119,316	395,965	92	38,669	339,294
export broiler meat including transit	(sl. weight in tons per year)	92,058	89,569	875,243	808	315,568	257,682
transit factor		0.80	-	0.80	-	-	0.40

Source: Gross domestic production broilers FAO STAT 2009; Import and export live broilers Netherlands: PVE (2010), Norway: StatBank Norway 2009, other countries: EUROSTAT DS-016894 2009; Import and export of broiler meat and meat products Norway: StatBank Norway 2009, other countries: EUROSTAT DS-016894 2009; Transit factor own estimation based on the fact that about 80% of consumed chicken meat comes from chickens raised in the own country.

# Campylobacter associated inputs

# Data on *Campylobacter* associated inputs appear in Table 29.

### Table 29. Campylobacter incidence and disease burden for DK, ES, NL, NO, PL and UK.

		,		,		
(cases per year)	17,698	1,760,064	96,044	6,333	1,546,234	328,990
(cases per year)	13,071	1,190,055	75,146	5,499	1,156,171	246,673
(cases per year)	27,256	2,806,168	126,709	7,226	2,135,261	488,964
(DALY per year)	476	47,308	2,582	170	41,605	8,836
(DALY per year)	340	31,199	1,952	141	30,253	6,422
(DALY per year)	743	76,346	3,480	203	58,295	13,263
	0.23	0.23	0.23	0.23	0.23	0.23
	0.23	0.23	0.23	0.23	0.23	0.23
	(cases per year) (cases per year) (cases per year) (DALY per year) (DALY per year) (DALY per year)	(cases per year)         17,698           (cases per year)         13,071           (cases per year)         27,256           (DALY per year)         476           (DALY per year)         340           (DALY per year)         743           0.23         0.23	(cases per year)         17,698         1,760,064           (cases per year)         13,071         1,190,055           (cases per year)         27,256         2,806,168           (DALY per year)         476         47,308           (DALY per year)         340         31,199           (DALY per year)         743         76,346           0.23         0.23         0.23	(cases per year)17,6981,760,06496,044(cases per year)13,0711,190,05575,146(cases per year)27,2562,806,168126,709(DALY per year)47647,3082,582(DALY per year)34031,1991,952(DALY per year)74376,3463,4800.230.230.230.23	(cases per year)17,6981,760,06496,0446,333(cases per year)13,0711,190,05575,1465,499(cases per year)27,2562,806,168126,7097,226(DALY per year)47647,3082,582170(DALY per year)34031,1991,952141(DALY per year)74376,3463,4802030.230.230.230.230.23	(cases per year)17,6981,760,06496,0446,3331,546,234(cases per year)13,0711,190,05575,1465,4991,156,171(cases per year)27,2562,806,168126,7097,2262,135,261(DALY per year)47647,3082,58217041,605(DALY per year)34031,1991,95214130,253(DALY per year)74376,3463,48020358,2950.230.230.230.230.230.23

Source: *Campylobacter* incidence and disease burden Mangen (2013); Attributable fraction to chicken meat cases and attributable fraction to chicken meat disease burden Havelaar et al. (2008) and Havelaar et al. (2009).

# Input for specific control measures

The data that have been collected for *Campylobacter* control measures are described below. The interventions were selected based on results of the risk factor analysis in CamCon WP1.

No depopulation schedule

No additional assumptions.

# Anteroom + hygiene barrier

- Number of houses per farm -> CamCon questionnaire (Høg et al., 2011)
- Number of broilers per house -> CamCon questionnaire (Høg et al., 2011)
- Number of houses with anteroom or/and hygiene barrier -> CamCon questionnaire (Høg et al., 2011)
- Anteroom: because the anteroom needs to be built separately unto an existing building, investment per m<sup>2</sup> was assumed to be 50 % higher than the normal investment per m<sup>2</sup> for a building (own assumption). Room was assumed to be 3 by 3 meter (own assumption).
- Hygiene barrier -> This was assumed to be a wooden beam (investment €100, own estimation) with 2 hours for installing the beam (own assumption).

### <u>New houses</u>

The percentage of farms to renew the houses was based on CamCon questionnaire (Høg et al., 2011). Demolition costs in the Netherlands were €10/m<sup>2</sup> (Vermeij et al., 2009), which is 5.3 % of

the investment per m<sup>2</sup> in a building. In the other countries this percentage is used for demolition costs.

# Drinkers without cup

Based on trails in the Netherlands and Denmark reported by van Harn et al. (2009) and Jørgensen (2012), an average was calculated: final life weight decreased with 2.45 % and feed conversion increased with 0.69 %, while the growing period remained the same. This was applied on all countries.

# Slaughter at 35 days

Farms that were practiced thinning, discontinue this.

For all countries: Weight at 35 days is 2021 g (Aviagen, Ross 308 standards, 2011). We assumed that in practice 95 % of this value can be reached (own assumption). Feed conversion is 1.56 at 35 days, based on a 0.0004 reduction of feed conversion per g of weight. Mortality decreases with 0.06 % point with each day less growing period (2007/43/CE). The revenue price is lower, due to lower breast meat yield (own estimation). The correction is revenue, price was: DK 0.03, ES 0.06, NL 0.04, PL 0.04 and UK 0.04 eurocent per kg live weight.

# Shorter downtime

The downtime in days for a farm was retrieved from the CamCon questionnaire (Høg et al., 2011). In Denmark, rodent control was assumed to costs  $\leq 1,074$  per year (Lawson et al.,2009). Rodent control costs in the other countries were adapted from this relative to farm size and labour costs. Disinfection costs were based on additional external labour of ten hours and the use of  $\leq 500$  of formaldehyde per disinfection. Costs of external labour were assumed to be  $\leq 50$  per hour in the Netherlands. Costs in the other countries were adapted from this relative to labour costs.

### Dedicated tools for each house

Investment of €300 in a separate set of basic tools, such as brooms, hammer, spanner, saw, screwdrivers, and Atomist for each house (own assumption). We assumed depreciation of 10.0 % and maintenance of 1.0 % (own assumption).

### Fly screens

This was only calculated for Denmark, since no data was available for other countries. A broiler house at a model farm was assumed to have either wall-inlet and roof-outlet ventilation or longitudinal ventilation with inlets on both side walls and fans at the end of the house. In the baseline the inlet strategy was used. For one broiler house the investment was estimated at 45.5 hours of labour for construction of fly screens and fixing them to the house and €1,100 investment in screens (based on €750 investment in materials, 32 hours of work for a house with 23,000 broilers). Maintenance was assumed to be 2.0 % of investment for brushing screens to remove pollen, plant material and dust and maintenance, and depreciation 6.67 % (life span of 15 years). All information from Lowman (2013: Personal communication R. Lowman, Ruff Biosecure Inc.).

In the sensitivity analysis the total screen strategy was used. Costs of this tighter screening strategy were €21.92 per 100 broilers investment in materials and €15.95 per 100 broilers investment in labour (personal information B. Hald (2013) about changes to Lawson et al.(2009)). Depreciation and maintenance was assumed to be the same as in the first screening strategy.

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# Appendix 1

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# Appendix 2

# Regression for censored data.

We used the model for log concentrations

 $y_i = a x_i + u_i, \text{ with } u_i \sim N(b, \sigma)$ (1)

with x and y concentrations to be associated.

Using (1), y is the variable for which we have censored data. The best values for a, b and  $\sigma$  were fitted to the data in Excel using the Solver.

Next,  $y_{exp}$  = a  $x_{obs}$  + b is calculated and then

 $P(Y_i=y_{obs})$  is calculated as Normdist( $y_{obs}$ ,  $y_{est}$ ,  $\sigma$ , true) and

 $P(Y_i < y_{obs})$  as Normdist( $y_{obs}$ ,  $y_{est}$ ,  $\sigma$ , false) or  $P(Y_i > y_{obs})$  as 1-Normdist( $y_{obs}$ ,  $y_{est}$ ,  $\sigma$ , false) (for censored data)

The log of all the P's is summed, and maximized for a, b and  $\sigma$ .